The Journal of Laboratory and Clinical Medicine

VOL. 26 FEBRUARY, 1941 No. 5

PROGRESS

FAMILIAL NONREAGINIC FOOD ALLERGY AS A PREDISPOSING CAUSE OF COMMON COLD

ARTHUR F. COCA, M.D., ORADELL, N. J.

THAT nonspecific influences may act as predisposing causes of common cold has been amply demonstrated in two of a brilliantly conceived series of studies on "Nonspecific Factors in Resistance" that have been carried out by Arthur Locke. These remarkable papers have had such scant attention in medical literature, and the conclusions reached in them are so directly applicable to the observations about to be described, that a summary of the outstanding pertinent results of Locke's findings is included here as follows:

SUMMARY OF LOCKE'S INVESTIGATIONS

1. Normal rabbits differ in the speed with which they can accomplish removal from the circulating blood of small numbers of intravenously injected type I pneumococci. Only those rabbits whose rate of removal of the injected pneumococci is faster than the rate of proliferation of the pneumococci survive the injection.

2. When normal rabbits are immersed in cold water until the body temperature has been reduced from the normal 102° to 103° F. to between 95° and 96° F., they differ according to the time required for a temperature recovery of 3° F.—"warming time." Those with fast recovery (warming times of 30 to 33) dispose of injections of small numbers of virulent type I pneumococci with a rapidity that permits a high percentage of survival. On the contrary, rabbits with a slow recovery (warming times longer than 45) are unable to remove the bacteria from the blood at a rate sufficient to assure survival.[1-4]

As Locke points out, these methods of study are not applicable to man, but in his study of common cold he has made use of two other methods with

*From the Lederle Laboratories, Inc., Pearl River, N. Y.

which he has obtained results that carry the same significance in principle as those of the rabbit experiments just described.

Locke determined the "oxygenation time" in a group of persons variously susceptible to common cold under conditions of physical stress which were produced by having the individual undertake a vigorous exercise of the muscles of the back, arms, and legs in a specially designed apparatus.[5] The "oxygenation time" represents the rate at which oxygen is used up by the individual when he exerts himself to the utmost in the apparatus; the figure thus obtained is corrected according to the estimated body surface of the person.

In the "optimal response" A the "oxygenation time" was in the neighborhood of 0.60; it was shorter, e.g., 0.40 to 0.44, in the "hyperresponse" C[1] and longer, e.g., 0.79 and 0.85, in the "hyporesponse" C.

Under this procedure Locke found that among those persons classified as A ("optimal response") 74 per cent reported less than two colds during the year, whereas among the C group only 5 per cent had had less than two colds during the year.

Locke found also that, regardless of the classification determined in the individual previous to the onset of a cold, the grading at the time the attack began was C, and the condition of the person was that of "physical exhaustion and shock which a grading of C connotes." He states: "Common cold has less similarity to infections of the type producing small-pox, typhoid and diphtheria than with infections of the type observed as a result of invasion from the lungs and intestines in dogs and rabbits during transient exhaustion[6] and shock.[7]"

In a more recent study[8] Locke has confirmed his general thesis that "low-grade infection (such as common cold) gains foot-hold because of impairment in the ability of the defense mechanisms to *function.*" In this study he has used a method which spares the individual the physical strain that was involved in the determination of the oxygenation rate. This method requires the person merely to inhale 5 per cent carbon dioxide for one to two minutes. The inhalation of 5 per cent carbon dioxide causes a "measurable, reproducible and characteristic increase in the depth of breathing."

Defining the "ventilation quotient" as the ventilation rate, noted under the influence of carbon dioxide, divided by the basal ventilation rate (B.V.R.), as observed when the person is merely lying at rest, Locke proposed the figure 1.29 as the optimal ventilation quotient of a normally resistant individual. A group of persons showing ventilation quotients within 5 per cent of this optimum showed a much lower incidence of common cold than did the group showing ventilation quotients diverging 10 per cent or more from that optimum. One may again sum up Locke's results with his two methods as follows: there is a state of impaired physiologic function, the nature and cause of which is unknown, that can be detected in the individual through two quantitative methods; this state of impaired physiologic function may be found to affect some individuals at one time but not at another. Those individuals in

whom the impaired function is not found are less susceptible to colds than are those in whom it is found, and the impairment is always present at the onset of a cold.

These findings indicate that susceptibility to common cold is not due to a lack of the mechanism of defense but to a nonspecific impairment of the ability of the mechanism to function.

For a certain purpose, unrelated to the present inquiry, I made in September, 1939, a survey of 825 employees of Lederle Laboratories in Pearl River, N. Y., with respect to the incidence of common cold. These persons were kept under periodic observation in the winter and spring of 1939-1940. Among the 825 individuals 99 reported having had no colds in the past three years. Locke in Pittsburgh finds 12 per cent of unselected persons free of colds.

Fifty-two of these cold-free persons were selected for further examination because of their ready accessibility and their intelligence. Fifty-one persons who had a history of at least one cold* annually were similarly selected for examination.

Each individual of these two groups was repeatedly questioned as to the presence of food allergy† in himself and in his immediate family. In a few instances, when the inquiry took place shortly after a meal, the pulse rate was observed. This latter evidence was helpful, of course, only when the rate was high enough to be considered probably food allergic. The results of this examination are shown in Tables I and II.

Among the group of 51 persons who were subject to colds, there were three (W. K., W. Kw., and A. W.) who presented no personal history of food allergy; however, the high pulse rate of 96 observed in two of these men one hour after breakfast marks them as most probably food allergic. N. G., J. H., and P. P. gave a bilateral negative family history of symptoms of food allergy, yet there is no doubt that all three are themselves food allergic. The strongest point of evidence of food allergy in C. C. is the bilateral family history of food allergy. The evidence of food allergy in H. H., B. R., and E. W. is weak.

Acknowledging the possibility that A. W., C. C., H. H., B. R., and E. W. were not food allergic, one may draw from these data the conclusion that a large proportion, probably about 90 per cent, of cold-susceptible persons are food allergic. Among the 52 persons who had had no colds in the seasons of greatest risk of 1939-1940, and who reported having had no colds in the previous two years, 31 reported a negative family history, as contrasted with three such persons among the cold-susceptible group. Two others had sisters or brothers all of whom were free from food-allergic symptoms. Five others

*If the question should arise as to whether some of the group of cold-susceptible persons were individuals with perennial allergic coryza, it can be pointed out that since the group was not self-selected, but chosen at random, not more than 2 per cent (at most one or two individuals) could be expected to be so affected. Incidentally, one of the cold-free group has had allergic coryza in the winter, which has been controlled by specific treatment. This employee never mistook her condition for common cold.

The cold-susceptible persons presented typical histories of an acute infectious coryza both as to clinical course and as to the characteristic change in the exudate.

†The expression "food allergy" throughout this paper refers only to familial nonreaginic sensitivity to food, a category which, according to evidence that will be presented in a later paper in this JOURNAL, must be separated from reaginic atopy (asthma, hay fever, atopic dermatitis).

TABLE I

INCIDENCE OF FOOD-ALLERGIC SYMPTOMS IN PERSONS SUSCEPTIBLE TO COLDS

NAME	COLDS EACH YEAR	PULSE RATE 1 TO 2 HOURS AFTER MEAL	HIVES	HEADACHES	HEARTBURN	INDIGESTION	CANKER SORES	DIZZINESS	DIARRHEA	NERVOUSNESS	NEURALGIA	PHYSICAL TIREDNESS	FAMILY HISTORY PARENTS OR SIBS	REMARKS
L. W.	2			x	x	x							M[1] negative; F[1] ?	
R. G.	1, not bad		x	x									M nervousness; F ?	
V. W.	x[2]	64		x		x	x	x		x			M headaches, indigestion	
W. M.	Oc[3]		x	Oc[3]									?	
D. S.	1			x		x	x		x			x	Unilateral	Has hay fever. Mother has all symptoms
J. L.	1			x	xx								?	
D. P.	1			x	x	x				x			?	
E. K.	1			xx								x	?	Milk causes constipation
N. G.	2	100		Oc		x					x[4]		Negative	
A. B.	Few, never bad					x	x	?				x	?	
J. B.	Some			Oc	x	x[5]	x			x			?	
L. B.	Many		x	x				x		x		x	Bilateral	Mother and father have same symptoms
E. B.	Some			x	x	x	x						Bilateral	Same symptoms excepting canker sores
J. C.	2	94	x	x			x						?	
H. C.	Colds	86		x		x					x		?	
F. C.	2-3			Oc					x[6]				M negative; F gastric ulcer	
J. C.	2	86		Oc						x			?	
H. E.	4		x	x									Bilateral	
L. G.	Some			x	x					x			Unilateral	
C. G.	Some	100		x				x	x	x	x		?	
J. G.	2+			x						x		x	Bilateral	

TABLE I—CONT'D

J. G.	Several		x	x	x	x						x	M headaches	
T. H.	Some	84		x								x	Bilateral	
J. H.	1		x[7]	Oc									Negative	
P. H.	Some			x	x								Parents ?; sister positive	Sister has headaches, heartburn, dizziness, nervousness
J. H.	3 or more			Oc	x								Unilateral	
F. J.	Some			x		x		x					Bilateral	
W. K.	1	96											Bilateral	
W. Kw.	1	96											M headaches, neuralgia	
R. K.	2?		x	x	x								Unilateral	
H. L.	Some[8]		x	Oc	x						x		M hives	
N. L.	Some		x										Bilateral	
E. M.	2			x	x		x						Unilateral	
H. M.	2 or more			x	x								?	
S. M.	Some	99	x										Unilateral	
P. P.	1	108									x[9]		Negative	
J. P.	Some			x		x							?	
A. R.	Some		x					x			x		?	
D. S.	Some		x					x					?	
T. S.	Some			x									M ?; F headaches	
M. S.	Some		x		x					x			?	
J. T.	Some			x						x			M heartburn, tiredness	
C. T.	Some			x		?	x	x	x	x	x	x	Bilateral	
E. V. L.	Several	100								±	±		F ?; M positive	Patient has dermographia and constipation
A. V. V.	Some		x	x		x							Bilateral	
H. Z.	2			x	x		x			x	x		M positive	
A. W.	2 or 3	72											?	Probably not food-allergic
C. C.	1	72				x							Bilateral	Questionably food-allergic
H. H.	1	68				x							M headaches	Questionably food-allergic
B. R.	Some				x								M asthma; sibs indigestion	Questionably food-allergic
E. W.	2			Oc						x			?	Questionably food-allergic

[1]M = mother; F = father.
[2]Sore throats.
[3]Oc = occasionally.
[4]Leg.
[5]Nausea.
[6]Crab.
[7]Orange.
[8]Also boils.
[9]Facial.

TABLE II

INCIDENCE OF FOOD-ALLERGIC SYMPTOMS IN PERSONS NOT SUSCEPTIBLE TO COLDS

NAME	COLDS LAST 3 YEARS	PULSE RATE 1 TO 2 HOURS AFTER MEAL	HIVES	HEADACHES	HEARTBURN	INDIGESTION	CANKER SORES	DIZZINESS	DIARRHEA	NERVOUSNESS	NEURALGIA	PHYSICAL TIREDNESS	FAMILY HISTORY PARENTS OR SIBS	REMARKS
B. P.	None												Unilateral	
F. P.	None												Negative	
A. R.	None												Negative	
S. R.	None												Negative	
H. S.	None												Negative	
W. S.	None							Oc[1]					?	High blood pressure
W. S.	None												Negative	
J. V.	None												Negative	
Mrs. J. V.	None												Negative	
D. V. H.	None												Negative	
W. W.	None												?	3 sisters negative
J. R.	None												Negative	3 sisters negative
J. K.	None	56									?		M[2] negative; F[2] ?	
E. M.	None												M negative; F ?	
A. S.	None												Negative	Asthma
M. M.	None			x[3]									Negative	Sore throat this winter; no cold
A. S.	None												M ?; F negative	
B. K.	None			x[3]									Negative	Father gassed twice
G. S.	None												Negative	Three years ago bronchitis and pleurisy
C. L.	None												?	3 sibs negative; wife and son (aged 18 years) headaches
M. A.	None												?	Hay fever
F. B.	None												?	
J. B.	None												M dizziness, nervousness; F negative	

TABLE II—CONT'D

E. B.	None												Negative	
J. B.	None												?	
M. B.	None												Negative	Sib hives
H. C.	None												Negative	
Dr. D.	None												Negative	
W. D.	None												Negative	
G. E.	None												Negative	
A. F.	None												Negative	
L. F.	None												Negative	
M. F.	None												?	
N. G.	None												Negative	
C. H.	None												Negative	
J. J.	None												Negative	
M. L.	None					?							Negative	
C. L.	None					?							?	Fullness on excessive eating
S. P.	None												Negative	
F. B.	None												Negative	
E. K.	None												?	Facial neuralgia 14 years ago
A. F.	None			x[4]									?	Questionable
M. S.	None	78		x[4]									?	Questionable case
													Sibs negative	
E. W.	None							x[5]		x[5]			Negative	Possibly allergic
A. K.	None			Oc[1, 5]									Negative	Questionably allergic
E. D.	None		x[6]	x[4]					x[7]				M Indigestion	Undoubtedly mildly allergic
D. S.	None	56	x[8]										?	Undoubtedly mildly allergic but avoids tomato
D. C.	None	64		Oc									Negative	Probably mildly allergic; formerly had colds
A. S.	None	92		Oc									Negative	Undoubtedly allergic
E. W.	None	86	x										Bilateral	Undoubtedly allergic
E. P.	None									x		x	M headaches; F ?	Probably allergic
J. G.	None			x[5]				x[5]		x[5]			Bilateral	Probably mildly allergic

[1]Oc = occasionally.
[2]M = mother; F = father.
[3]With eyestrain.
[4]Very seldom.
[5]At periods.
[6]Formerly, not now.
[7]Colitis one summer.
[8]Only from tomatoes.

reported a negative history in one parent. Twelve were unable for one or another reason to inquire of either parent about the matter, and only two gave a bilaterally positive family history of food allergy, as contrasted with eleven such persons in the cold-susceptible group.

The last seven of the cold-free group are no doubt food allergic, but mildly so, excepting possibly A. S. and E. W. The preceding four are questionable with regard to food allergy.

The comparison of the data in Tables I and II hardly permits any doubt as to the important role of food allergy in predisposing the person to common cold. Granting this conclusion, one can now appreciate fully Locke's application to his problem of the scientific method, in both design and technique, and also the accuracy of his deductions.

Throughout his study Locke seems to have been quite in the dark as to the nature of the original causes of the "nonspecific factors" of whose existence he was collecting such convincing evidence. Although the thought of allergy as a possible nonspecific influence evidently crossed his mind, it seems to have been set aside, no doubt because he was not aware of the large incidence of that state and had no method of detecting its presence.

Particularly illustrative of his correct insight is his forthright assertion: "Probable nonspecific predisposition to common cold, at the time of test, is not identical with the probable future incidence." In the light of the results of our own investigation, this statement may be paraphrased as follows: the food-allergic state, which usually predisposes to common cold, may be present at the time of test in some persons who are not cold susceptible (see last 7 cases in Table II).

For the question: What is the nature of the nonspecific predisposing cause of common cold? there is a suggestion that arises out of a very commonly observed phenomenon in food-allergic persons: namely, the rapid increase and equally rapid loss of weight which sometimes is noticed at the onset of allergic headache and as the attack passes off. Some observers have reported that the loss of weight has been associated with an increase in the excretion of urine.

I have noticed this phenomenon in several persons with food allergy whose symptoms were relieved by avoidance of the allergenic foods. In two persons, J. G. and A. P., urticaria was an outstanding symptom. After the allergenic foods were identified and eliminated, there was a permanent loss of several pounds in weight, although the diet was ample and well balanced and the general health was decidedly improved in both individuals. The rapid loss of weight in these cases is, of course, due to the withdrawal of fluid from the edematous tissues similar to that described by von Pirquet and Schick in the recovery from serum disease. This phenomenon is not limited to cases of urticaria or so-called angioneurotic edema. The allergic infiltration of fluid into tissues is usually so distributed as not to be grossly perceptible.

From the foregoing considerations it is seen that in those cold-susceptible persons in whom food allergy is a predisposing cause of the susceptibility, the

"nonspecific factor" of Locke may be the effect of allergic edema. This suggestion has some support in earlier published observations, as interpreted by Hoelzel[9] and others. A number of investigators[10-15] have reported that the incidence of colds is reduced by restriction of carbohydrate consumption. Hoelzel,[10] McQuarrie,[12] and Higgins[13] attributed the reduction in the number of colds to an observed lessening of tissue hydration.

There must be other predisposing causes of susceptibility besides food allergy. This conclusion seems forced by the fact that, although between 20 and 25 per cent of white families are free of food allergy,* only 12 per cent are quite free of common cold, and a few of these are food allergic.

SUMMARY

Evidence is offered of the importance of food allergy as a predisposing cause of common cold.

REFERENCES

1. Locke, A.: Lack of Fitness as the Predisposing Factor in Infections of the Type Encountered in Pneumonia and in Common Cold, J. Infect. Dis. **60**: 106, 1937.
2. Locke, A.: Nonspecific Factors in Resistance. I. Capacity to Sustain Effective Circulation, J. Immunol. **36**: 159, 1939.
3. Locke, A., and Main, E. R.: Nonspecific Factors in Resistance. II. Ability to Withstand Shock, J. Immunol. **36**: 173, 1939.
4. Locke, A., Main, E. R., and Mellon, R. R.: Nonspecific Factors in Resistance. III. Capacity for Retarding Bacterial Proliferation, J. Immunol. **36**: 183, 1939.
5. Locke, A.: Nonspecific Factors in Resistance. IV. The Problem of Common Cold, J. Immunol. **36**: 365, 1939.
6. Bailey, G. Howard: Accessory Etiologic Factors of Respiratory Infection in Rabbits. II. The Effect of Fatigue on the Susceptibility of Rabbits to Intranasal Infection With Type I Pneumococcus, Am. J. Hyg. **9**: 192, 1929; Boycott, A. E., and Price-Jones, C.: Experiments on the Influence of Fatigue on Infection, J. Path. & Bact. **29**: 87, 1926.
7. Boone, T. H., Chase, E. M., and Brink, H. E.: Intestinal Absorption of B. prodigiosus, Proc. Soc. Exper. Biol. & Med. **29**: 113, 1931.
8. Locke, A.: Nonspecific Factors in Resistance. V. Carbon Dioxide Tolerance, J. Immunol. **39**: 441, 1940.
9. Hoelzel, F.: Diet and Resistance to Colds, Science **86**: 399, 1937.
10. Hoelzel, F.: Nutritional Edema and Its Relation to the Incidence of Common Colds, Proc. Soc. Exper. Biol. & Med. **25**: 454, 1928.
11. Hoelzel, F.: A Relation Between the Incidence of Common Colds and Nutritional Hydration, Science **68**: 301, 1928.
12. McQuarrie, I.: Protein Metabolism of Children on Diets Extremely Low in Carbohydrates, J. Nutrition **2**: 31, 1929.
13. Higgins, H. L.: Some Physiological and Clinical Effects of High Fat Feeding, New England J. Med. **203**: 145, 1930.
14. Orr, J. B., and Gilks, J. L.: Studies on Nutrition: The Physique and Health of Two African Tribes, Medical Research Council, Special Report No. 155, 1931.
15. Paton, J. H. P.: Relation of Excessive Carbohydrate Ingestion to Catarrhs and Other Diseases, Brit. M. J. **1**: 738, 1933.
16. Pickrell, K. L.: Effect of Alcoholic Intoxication and Ether Anesthesia on Resistance to Pneumococcal Infection, Proc. Soc. Exper. Biol. & Med. **38**: 265, 1938.

*This figure is taken from a survey conducted under the sponsorship of the Board of Health of Oradell, N. J.

CLINICAL AND EXPERIMENTAL

THE DETERMINATION OF BLOOD VELOCITY BY LOBELINE*

FRANK V. PICCIONE, M.D., AND LINN J. BOYD, M.D., F.A.C.P.
NEW YORK, N. Y.

DURING an investigation of the pharmacologic properties of lobeline, the "circulation time" of 95 individuals was determined by this substance. In this communication discussion will be limited to directing attention to a new method of ascertaining the end point of the test and to reporting an additional series of patients studied by this means.

The original contribution of Teplov and Sor,[1] based upon the study of 165 patients, indicated that lobeline provided an objective method for the determination of circulation time. The end point (cough) was striking and brief, the procedure simple, the drug free from toxicity, and the result susceptible to graphic registration. When less than 0.03 to 0.04 mg. of lobeline (or less than 0.08 to 0.12 mg. of synthetic lobeline) per kilogram was employed intravenously, respiration was altered at times; with this dose they noted brief apnea, a *cough*, and then transient polypnea. Cough was elicited in 60 to 70 per cent of the patients; the percentage of successful results was increased by repetition of the test after 15 to 20 minutes with a slightly larger dose. If cough still failed to occur, the polypnea was registered by a pneumograph in order to obtain an objective end point.

Volgin and Stanojevič[2] alone observed 49 patients, and with Djordjevic[3] they studied 80 individuals. Stanojevič[4] participated in 500 determinations on 150 patients. Lobeline was used exclusively, and only 7 patients failed to respond with cough; the dosage was individualized but did not exceed 0.07 mg. per kilogram. The circulation time amounted to 10.3 seconds in the average normal adult (Teplov and Sor, 13.4 seconds), to 12.4 seconds in miscellaneous patients; in patients with cardiac compensation it was 13.4 and in those with cardiac decompensation it was 30.2 seconds (Teplov and Sor, 40 seconds). On the basis of 140 determinations Evzlina[5] concluded that the method was objective, free from unpleasant concomitant manifestations, uniform in its results, and harmless to patients. Trimarchi,[6] employing lobeline up to 0.18 mg. per kilogram of body weight, reached a similar conclusion. Large amounts seemed necessary in some cases of auricular fibrillation; when the maximal dose was employed, precordial pain and dyspnea developed in some cases, lasted for a few seconds, and vanished without sequelae.

During preliminary experiments arranged to familiarize ourselves with the method, we noted that patients often commented upon a peculiar sensation in the throat ("tickling"); this may or may not be accompanied by cough.

Twenty males whose ages varied from 13 to 64 years and whose weights ranged from 90 to 175 pounds comprised this group. Most of them were suffering from gonococcal urethritis and a few from pes planus. None of them had cough as a symptom. The tests

*From the Department of Medicine (Metropolitan Hospital Service), New York Medical College, Flower and Fifth Avenue Hospitals.
Received for publication, November 10, 1939.

were conducted under basal conditions and at the same time of day. Patients imagined they were receiving some therapy. Each of them, regardless of age or weight, received 3 mg. of lobeline intravenously. After the injection they were asked whether they felt anything. If nothing occurred, the test was repeated with 6 mg. of lobeline.

When 3 mg. of lobeline were administered, 9 patients had tickling in the throat, 6 had cough, and 5 had no response. When 6 mg. of lobeline were given to the 9 people who had tickling, 6 responded with cough and 3 with more pronounced tickling. Among the 5 with no response, 3 developed tickling and 2 cough with the larger dose. In short, 15 of 20 patients had an objective response, and the remainder had a reaction which made the end point easily recognized. No ill effects followed.

These results suggest that the intravenous injection of lobeline in doses of from 3 to 6 mg. provides a dependable reaction for the objective determination of circulation in 75 per cent of patients. Employment of an additional subjective symptom practically eliminates negative responses when the larger dose is given.

The circulation time of 75 individuals suffering from various illnesses was then determined.

Alpha-lobeline was employed exclusively. Since 1 c.c. ampoules contain 0.01 Gm., 0.1 c.c. corresponds to 1 mg., and no difficulty is encountered in calculation of dose. Moreover, the volume of the solution is small and the time required for injection is short. Although 5 mg. will usually elicit a response, 3 to 7 mg. were employed in accordance with the weight. (Trimarchi has injected 11 mg. in persons with severe cardiac disease.)

Patients were asked to lie as flat as possible, to relax, and not to hold the breath. The stomach must be empty. The purpose of the test is fully explained to dispel any fear. A tourniquet may be applied to the arm to assist in visualization of the vein, but it should be removed prior to the injection. The injection time should not exceed two seconds. The starting point of the time was placed at the moment the plunger struck the bottom of the syringe; the end point was considered the elevation of the hand or the cough. An ordinary stop watch was employed. A 23-gauge needle, 1 inch in length, was found to be most suitable. No patient experienced any pain; a few times some of the solution spilled into the paravenous tissues but no inconvenience or symptoms resulted. No patient objected to a repetition of the test. Three patients violated the rule of an empty stomach and ate fruit one-half hour before the injection. One did not respond at all; the second responded after fifty seconds, although an eight-second circulation time was obtained on the following day; the third, who had a decompensated cardiac involvement with a prolonged circulation time, showed excessive prolongation (more than one minute), then coughed and vomited.

With small doses (3 to 4 mg.) the rate and depth of respiration may change. However, usually this cannot be employed as an end point since the first alteration of respiration may not be recognizable. Tracings of the respiratory excursion can be secured, but a necessity for any special device defeats general employment of the test.

A peculiar sensation in the throat precedes or accompanies the cough. The occurrence of this sensation may be accompanied by a grimace. The sensation is described in various manners, but usually as tickling or "something coming up quick in the throat." At all events it is perceived as something unusual. Patients were instructed to raise their hand as soon as they felt anything; usually cough occurred while the hand was being elevated. If there is an interval between the two phenomena, not more than one or two seconds separate them.

The cough resembles the explosive cough caused by the inhalation of smoke or dust. It is not severe nor does it distress patients in any way. Individuals suffering from bronchiectasis, pneumonoconiosis, or pulmonary tuberculosis, said that the cough was un-

usual and sounded different. Occasionally the patient asked for a sip of water "to fix the throat" which "felt dry." Three patients noted moderate dyspnea; one patient reported a sensation of choking, although no dyspnea was observed. Many individuals experienced an improvement of respiration, were pleased with the injection, and the momentary cough was attributed to nervousness.

No members of the other groups to be reported were normal, but many of them sought treatment for trivial conditions, such as pes cavus and bursitis, which may be regarded as having no bearing on circulation. Physical examination of this group yielded normal findings in the lungs and cardiovascular system. Patients with important affections not involving the heart or respiratory system were also examined. They were considered normal from the cardiovascular standpoint.

In many the venous pressure was estimated by the degree of engorgement of the veins of the neck and the level at which the arm veins collapsed upon elevation of the limb. Exact measurement was regarded as unnecessary. Vital capacity was determined by the usual apparatus, but it did not constitute a part of the test.

Group 1.—Six patients, 5 females and one male, formed this group. Their ages ranged from 22 to 52 years. Four had secondary anemia and tuberculosis, one had a secondary anemia and subacute bacterial endocarditis without cardiac decompensation, and one had pernicious anemia. The hemoglobin estimations in the group ranged from 60 to 66 per cent; the red blood cells did not vary more than 200,000 from three million.

The circulation time in this group of patients who had anemia varied from three and one-half to eight seconds. The two shortest circulation times in the entire series occurred in this group.

Group 2.—Twenty-three patients, 13 males and 10 females, were placed in this group. Their ages varied from 13 to 60 years. Only one patient had a cardiovascular anomaly (cirrhosis of the liver, pulmonary emphysema, and possible beriberi heart. There was some peripheral edema. His circulation time was eight seconds).

The average circulation time in this group of patients who had no anemia, no cardiovascular disease (one exception), but definite illnesses, was 8.3 seconds. One individual (tonsillitis) had a circulation time of twenty seconds but no demonstrable cardiovascular involvement. Individuals with manifest thyrotoxicosis (two observations) had times of five and nine seconds. With the one exception noted the extremes of this group were five to twelve seconds.

Group 3a.—Two males and one female, whose ages varied from 22 to 62, suffered from mild, moderate, and severe pulmonary fibrosis, associated with pneumonoconiosis; two of them had electrocardiographic alterations as well. The circulation times of these patients ranged from six to nine seconds. The patient with the lowest vital capacity had the shortest circulation time.

Group 3b.—Three male patients, whose ages were 32, 39, 41, had pneumothorax (one pyopneumothorax). The circulation times ranged from seven to fourteen seconds. The individual with the longest circulation time had a Peabody factor only slightly inferior to that of the patient with a circulation time of seven seconds. There seemed to be no relationship between the Peabody factor and circulation.

Group 3c.—Eleven patients, 8 males and 3 females, comprised this group. Their ages varied from 23 to 79. They suffered from various chest diseases, such as pneumonia, pulmonary

malignancy, and caseous pneumonic tuberculosis. The circulation times ranged from six to ten seconds. The patient with the longest circulation time had pneumonia. The patients with the lowest Peabody factors (3.6, 3.3) had shorter circulation times than a patient with a much higher Peabody factor (5.6). Although cough and dyspnea were present in pulmonary malignancy, the circulation time was eight seconds.

Among 17 patients presenting various diseases of the lungs, the circulation time varied from six to fourteen seconds. Dyspnea of pulmonary origin did not prolong the circulation time.

Group 4a.—Three males and 2 females were placed in this division. Four suffered from rheumatic heart disease, and one from syphilitic aortic valve insufficiency. One patient had cardiac compensation (ten-second circulation time). Two patients had auricular fibrillation (circulation times of twenty-five and twenty-eight seconds). In the 4 patients with cardiac decompensation the extremes of circulation time were from sixteen to twenty-eight seconds.

Group 4b.—Six males and 7 females, whose ages ranged from 32 to 71, were placed in this class. All of them had hypertension and some had suggestive evidence of coronary artery disease. The circulation times varied from twelve to thirty-five seconds. The patient with the shortest circulation time was able to lie perfectly flat, but she had some dyspnea on walking (very obese woman). Patients with circulation times of fourteen to seventeen seconds "had to sleep with pillows," were "short of breath at night," or had dyspnea on walking. Those with circulation times of seventeen to thirty-five seconds had definite cardiac decompensation.

Group 4c.—This division contained 11 patients, 9 males and 2 females. The ages varied from 44 to 83. They all had marked evidence of coronary artery disease. Two had auricular fibrillation, one had angina pectoris, and two had survived coronary thrombosis. The circulation times in the group ranged from eleven to forty seconds. The group with the eleven- to sixteen-second circulation time was composed as follows: 2 without symptoms (the oldest patients) gave responses at eleven and fifteen seconds; one with recent coronary thrombosis, without evidence of cardiac decompensation, at thirteen seconds; one with recently recovered cardiac compensation, at fourteen seconds; one with slight dyspnea, at sixteen seconds. The remainder obviously had cardiac decompensation and their circulation times ranged from twenty to forty seconds.

Patients with cardiac decompensation showed a definite prolongation of the circulation time. Circulation times above twenty seconds were always associated with cardiac decompensation, but one patient with unproved but probable cardiac decompensation (group 2) had a circulation time of eight seconds. On the contrary, patients with a wide assortment of diseases (one exception) did not have circulation times above sixteen seconds. This exception appears quite important and suggests that exceptions occur in both directions, namely, normal circulation time in rare cases despite cardiac decompensation and prolonged circulation time despite the absence of cardiac decompensation. These exceptions imply that this (and other similar tests) are far from infallible and cause one to doubt their application to the diagnosis of cardiac decompensation. In short, one wonders whether they are sufficiently delicate to be suggestive in early cardiac decompensation and whether they are necessary in manifest cardiac decompensation.

Nevertheless, repetitions of the test showed increased velocity of the blood stream as improvement occurred in cardiac decompensation; when the circulation time remained status quo the general situation persisted unchanged. For

example, the circulation times at two-week intervals in one individual with cardiac decompensation were thirty, twenty-two, fifteen seconds, respectively, as he improved. One patient with cardiac decompensation had a circulation time of twenty-five seconds; one and two weeks later the same reading was obtained and no recognizable change occurred in the situation during the interim. Similar results were obtained in numerous other individuals.

Volgin and Stanojevič[2] compared the circulation time obtained with lobeline with that secured by decholin and saccharin. They found the circulation time in all groups was longer with the decholin and saccharin and that variations were greater within each of the various groups, i.e., among normal individuals and among patients with cardiac decompensation. The longer time might be partly explained by the difference in the mechanism, that is, decholin and saccharin are arm-tongue methods, whereas lobeline is an arm-carotid sinus method. The results obtained by decholin and saccharin are similar, and the discrepancy in comparison to lobeline is greatest in patients with cardiac decompensation. A study of lobeline and calcium gluconate indicates that the circulation time with the former is shorter than with the latter. In ten experiments the lobeline time was one to seven seconds shorter than the calcium gluconate time in nine patients; the calcium gluconate time was shorter than the lobeline by two seconds in one patient.

A review of all the results obtained indicates that a sharp line cannot be drawn between individuals whose circulation is normal and those with cardiac decompensation by means of the circulation time. Nevertheless, several observations seem worthy of mention.

Many patients with pulmonary tuberculosis, bronchiogenic carcinoma, pleurisy, pneumothorax or anemia, exhibited dyspnea at rest or upon exertion. The circulation time was not prolonged in any of these individuals as long as cardiac decompensation was absent. Accordingly, it seems that the test might have value in assisting to differentiate between dyspneas of cardiac and pulmonary origin.

The degree of edema and the height of the venous pressure did not parallel the circulation time. In two patients who suffered from hypertension and who had marked anasarca and increased venous pressures (three plus), the circulation times were seventeen seconds in one and thirty-four seconds in the other. One patient with cirrhosis of the liver and possible beriberi heart had edema of the abdominal wall and of the lower extremities and increased venous pressure (one plus), although the circulation time was eight seconds. The circulation time does not seem to be correlated with the degree of failure of the right side of the heart, but bears a relation to left-sided decompensation. Since the occurrence of edema and the increase of venous pressure coincide with the failure of the right ventricle (other causes being excluded), the height of the venous pressure bears no relation to the circulation time. In fact, patients with normal venous pressures showed a greater circulation time than those with very high venous pressures (two and three plus). One individual with the high venous pressure (three plus) had the same prolongation of circulation time (thirty-four seconds) as another with a slight increase (one plus).

A vital capacity as low as 550 was found in a patient with a circulation time of six seconds. The patient had pulmonary fibrosis but no cardiac failure.

Another patient with a vital capacity of 3,400 and a Peabody factor of 7.5 showed a circulation time of eighteen seconds; still another individual with a vital capacity of 1,200 and a Peabody factor of 2.7 had a circulation time of eight seconds. Reductions of vital capacity not of cardiac origin do not seem to influence the circulation time.

Circulation times between three and thirteen seconds were recorded in patients with no myocardial failure. Circulation times of twenty-four seconds or more occur in persons with cardiac decompensation. Normal persons with compensated heart disease or with incipient myocardial failure had circulation times of fourteen to twenty-four seconds. The longest circulation time in a person without cardiac involvement (tonsillitis) was twenty seconds; the average for all those without cardiac disturbance was eleven seconds. The shortest circulation time in the presence of indisputable myocardial failure was thirteen seconds, the longest was forty seconds, and the average was twenty-seven seconds. Accordingly, the method cannot be employed to determine the existence of circulatory failure. However, it may assist in the differential diagnosis of dyspnea and in the determination of the therapeutic progress of a patient.

It does not seem advisable to enter into a discussion of the mechanisms thought to be involved in the lobeline test since the problem has not been completely solved. However, the weight of evidence[7] strongly favors the participation of the carotid sinus in the response. Accordingly, the lobeline and sodium cyanide methods for the determination of the circulation time are closely allied.

SUMMARY

Lobeline was employed for the determination of the circulation time in 95 patients. The absence of intolerance, toxicity, or cumulation noted by other observers found further confirmation. A new subjective response was observed; this "tickling" sensation as the end point may be employed when cough is present and might be confused with the lobeline cough; it also makes the test applicable without recourse to special devices and permits a definite result to be obtained in practically all cases.

The objectivity of the lobeline test endows it with certain definite advantages over some other substances employed for the same purpose. However, in determination of the existence of cardiac decompensation, it possesses those disadvantages which are intrinsic to any "circulation time" method. As an objective means of differentiating dyspnea of left ventricular origin and that of pulmonary origin, and as an aid in following the course of therapy, it may provide some very useful information.

REFERENCES

1. Teplov, J., and Sor, V.: Terapevticeski Arch. **13**: 57, 1935. Cited after Stanojević.[4]
2. Volgin, O., and Stanojević, L.: Vergleichende untersuchungen der Bilirubinämie und Kreislaufzeit bei Dekompensation des Herzens, Klin. Wchnschr. **17**: 569, 1938.
3. Volgin, O., Stanojević, L., and Djordjevic, B.: Odredivanje brzine krvotoka lobelinom, Medicinski Pregled. **11**: 90, 1936.
4. Stanojević, L.: Die Bestimmung der Kreislaufzeit mit Lobelin, Ztschr. f. Kreislaufforsch. **30**: 521, 1938.

5. Evzlina, M. M.: Lobeline in Determination of Velocity of Blood Circulation, Vrach. delo. **20**: 113, 1938.
6. Trimarchi, E.: Sul metodo della lobelina per la misura della velocità circolatoria, Riforma med. **54**: 1407, 1938.
7. Trimarchi, E.: Sull'azione farmacodinamica della lobelina, Rassegna di fisiopat. clin. e terap. **10**: 621, 1938.

REPORT OF A CASE OF CONGENITAL ABSENCE OF THE SPLEEN AND CHRONIC AMNIOTIC FLUID PNEUMONIA

RICHARD L. NELSON, M.D., F.A.A.P., AND D. R. VENABLE, A.B., M.D.
WICHITA FALLS, TEXAS

THE congenital anomaly herein reported is not common, judging from the comparatively few such cases found in the literature. During the past ten years we were able to find only five reported cases of congenital absence of the spleen, and only one of these was in an American journal, the interesting account by M. S. Peterman in the *Journal of the American Medical Association* for October 8, 1932, of a double anomaly, the absence of both spleen and left kidney. The history of apparent constitutional inferiority that the author gave in this case is very similar to the history of the fourteen-month-old boy upon whom we performed a necropsy.

The child was resuscitated with considerable difficulty at birth, having aspirated a large amount of mucus and amniotic fluid. Examination twelve hours after delivery revealed a listless, cyanotic baby, with a weak cry and an inability to nurse, although he weighed nine pounds and was a full-term child. Coarse râles were heard over both lungs, and he seemed to breathe with considerable difficulty. The heart was normal to auscultation and percussion. At three weeks of age an x-ray examination of the chest showed marked increase in pulmonary markings, but no enlargement of the thymic shadow. The heart was normal in size, shape, and position. At this time no râles could be heard over the lungs, but the infant continued to be cyanotic; he had little appetite, and was quite feeble. When he was six months old, he developed an attack of bronchopneumonia and was acutely ill for several days. He recovered from this but continued to be marasmic and the peculiar cyanosis persisted. He was not seen again until three days prior to his death, when the mother noticed that his left foot was cold and clammy and showed a bluish discoloration. He had some fever at this time. He became rapidly worse and developed a temperature of 102.6° F., rapid respirations, and rapid pulse rate. The heart was enlarged to the left, with a heaving impulse and a blowing systolic murmur at the apex. The liver margin could be felt one and one-half fingerbreadths below the costal margin. The spleen could not be palpated. The lungs were clear. An electrocardiogram showed right axis deviation and tachycardia. The lower third of the left leg and the left foot were cold and purple, with a sharply marked border indicating beginning gangrene. No pulsation could be felt in the anterior tibial artery. Blood cultures and other indicated laboratory procedures were omitted out of deference to the parents' wishes, since it was so evident that death was imminent. Local and supportive

treatment were of no avail, and the child died the following day. Just prior to death the temperature rose to 106° F., and the pulse rate was so rapid it could not be counted.

The necropsy showed a fairly well-nourished infant. The lower third of his left foot and his left leg were gangrenous due to thrombosis of the blood vessels. The lungs appeared firmer than normal and microscopic sections showed a peculiar condition in that many of the alveoli were obliterated by fibrous connective tissue; the walls of the functioning ones were very thick, probably the result of the amniotic fluid pneumonia that the infant had at birth. The heart was greatly dilated and the pericardial sac contained about twice the normal amount of fluid which was blood-stained but not purulent. There were fresh vegetations on the mitral valves which also showed other evidence of acute inflammation. The other valves were normal. There was no evidence of any congenital anomaly of the heart which might have accounted for the cyanosis that was present throughout the child's life. A large ante-mortem clot, which was firmly adherent to the chorda tendineae and showed beginning organization, was found in the left ventricle. There were fibroblasts present as well as polymorphonuclear leucocytes and some lymphocytes. The liver was large and congested, and the liver cells showed albuminous and fatty degeneration. The kidneys showed similar changes but to a much less degree. The gastrointestinal tract and pancreas were normal. A thorough search failed to reveal a spleen, although all the viscera were removed from the abdomen. An unsuccessful attempt was made to locate a splenic anlage by following up the branches of the celiac artery, but no such vessel was present. Acute bacterial endocarditis with emboli to the blood vessels of the lower third of the left leg and the left foot and the resulting thrombosis were the immediate cause of the death of the infant. Just what part, if any, the congenital absence of the spleen could have played in the production of the marked cyanosis that persisted throughout this child's brief life is an interesting speculation. Since the spleen is such an important organ in the hematopoietic system, it is conceivable that its congenital absence might have exerted a profound and adverse influence upon the circulatory system or, for that matter, upon the whole constitutional well-being of the child.

THE PHARMACOLOGY OF SILICA*

Effects and Mechanism of Action of Fatal Intravenous Doses of Silicic Acid

Walter Modell, M.D., and Charles Salzman, B.S., New York, N. Y.

THE study presented here is part of a larger project on the nature of silicosis. The exact form of silica which produces silicosis is not known, but there is evidence that silicic acid may play a role as an intermediary chemical agent in the development of the disease.[1] We shall deal here with the results of an investigation of the pharmacology of silicic acid† and give special consideration to the following: I. Toxic and lethal dose. II. Systemic effects. III. Mechanism: (a) role of blood clot formation and (b) site of action. IV. Significance of the colloidal nature of silicic acid.

METHOD

Cats were used in our experiments. Silicic acid sol was the only form of colloidal silica examined. It was prepared by dissolving suitable amounts of sodium silicate in water and treating with an excess of hydrochloric acid. This was dialyzed against water until free of chlorides and then concentrated to somewhat more than 1 per cent by evaporation with heat under reduced pressure. The silicon dioxide content was determined gravimetrically by drying and blast burning. The solution was then diluted to 1 per cent‡ and sufficient sodium chloride was added to make an 0.85 per cent saline concentration. A small amount of hydrochloric acid was added to retard the development of gel. The solutions were stored in a refrigerator when not in use. All solutions were slightly opalescent at the outset but no gel was visible macroscopically. The pH of each batch was determined electrometrically. This varied with each specimen. The same batch of silicic acid was used in each series of experiments.

The cats were anesthetized with sodium amytal by the intravenous or the intraperitoneal route, usually the former in doses of 40 mg. per kilogram.§ In most experiments kymographic records were made of the effects of silicic acid on blood pressure, heart rate, and respiration, although some observations were made on intact unanesthetized animals. Electrocardiographic records were made

*From the Department of Pharmacology, Cornell University Medical College, New York.

This study was made under the provisions of a Works Progress Administration Project sponsored by the Department of Health of New York City, and carried out by the Cornell University Medical College, New York Hospital, and the Industrial Hygiene Committee of the New York Tuberculosis and Health Association acting as cooperating agencies.

Received for publication, December 5, 1939.

†The terms silicic acid and colloidal silica will be used interchangeably.

‡Batches 1 and 6 were made up as 0.75 per cent and 2.0 per cent, respectively. The amount of silicic acid is always expressed in terms of silicon dioxide content.

§All doses are considered in relation to body weight, but for purposes of convenience the phrase, "per kilogram," will be omitted.

in some experiments. Silicic acid was always administered intravenously. In order to prevent the formation of intravascular clots and clots in the recording system, an anticoagulant dye was used in all but a few stated experiments. This procedure is discussed later.

All animals were examined after death. Sections of tissue were preserved for microscopic examination, but the results of this examination will form part of another report.

EXPERIMENTAL

I. Toxic and Lethal Dose.—The lethal dose of silicic acid was determined by continuous intravenous injection. The injection was stopped when blood pressure fell to about a level of 100 mm. Hg, since such falls were almost always fatal. However, more silicic acid was administered if necessary. The animals were anesthetized and the blood pressure was recorded from the carotid artery. The rate of intravenous injection was varied to determine what role the rate of administration might play in the toxicity of silicic acid.

Great variability of the fatal dose of silicic acid within each batch and between batches was a prominent feature. The average lethal dose of batches varied from 15 mg. to 193 mg. The smallest single fatal dose was 5 mg. and the largest was 284 mg. The lethal dose within each batch, however, remained in the same general range. Thus, in the batch in which 5 mg. was lethal in one case, the largest dose was 20 mg., while in the batch in which 284 mg. was the largest dose, the smallest dose was 142 mg. Lethal doses of the same specimen of silicic acid varied as much as 100 per cent on the same day, ruling out the possibility that a change in the silicic acid had occurred. The toxic dose (i.e. the dose necessary to produce a perceptible fall in blood pressure) was as variable as the lethal dose; generally it was about 60 per cent of the lethal dose.

TABLE I

TABULATION OF pH OF SPECIMENS OF SILICIC ACID AND THE LETHAL DOSE

BATCH NO.	pH	AVG. L. D. MG./KG.	NO. CATS
4	1.53	165	8
1	1.55	15	2
7	1.60	48	5
7a	1.60	53	9
2	1.78	15	3
3	1.87	30	2
6	2.40	152	10
5	2.65	193	14

The pH of the different batches of silicic acid varied between 1.53 and 2.65. Table I indicates the absence of correlation between the lethal dose and the pH. In addition, preliminary experiments with salt solutions, to which hydrochloric acid had been added to produce the same pH as that of the silicic acid specimens used in our experiments, indicated that the acidity itself was without any effect in the amounts used.

From the results in Table II it appears that the more rapid injections required larger amounts of silicic acid to produce death, but this may be due to a latent period in the development of the effects of silicic acid, so that the end

TABLE II

TABULATION OF AVERAGE RATE OF INJECTION AND THE LETHAL DOSE

BATCH NO.	AVG. RATE INJECTION MG./KG./SEC.	AVG. L. D. MG./KG.	RANGE OF RATE INJECTION MG./KG./SEC.	NO. CATS
7	0.25	48	0.19-0.37	5
3	0.26	30	0.17-0.35	2
1	0.44	15	0.33-0.54	2
2	0.53	15	0.20-0.83	3
4	1.33	165	0.06-3.91	8
5	1.40	193	0.47-2.37	14
7a	2.03	53	0.96-3.33	9
6	2.53	152	0.70-4.00	10

TABLE III

TABULATION WITHIN BATCHES OF SILICIC ACID OF THE RATE OF INJECTION AND THE LETHAL DOSE

RATE INJECTION MG./KG./SEC.	LETHAL DOSE MG./KG.
Batch 6—pH 2.40	
0.7	85
1.6	146
1.6	190
1.8	106
1.8	211
2.6	220
3.6	134
3.6	180
4.0	88
4.0	159
Batch 7—pH 1.60	
0.19	69
0.21	37
0.24	58
0.25	30
0.37	44
Batch 7a—pH 1.60	
0.96	43
1.26	63
1.28	41
1.33	56
1.54	54
2.34	82
2.92	35
3.27	49
3.33	50

Note: Batches 7 and 7a are the same specimen of silicic acid. The division into groups represents slow and rapid injections.

point was passed in the titration. In Table III, however, in which data for the lethal dose and the rate of administration for all experiments with two batches of silicic acid are given, there appears to be no significant correlation with the injection rate.

II. Systemic Effects.—The effects produced by all batches of silicic acid were qualitatively the same. In unanesthetized animals it produced an acceleration of respiration, which in the case of fatal doses was followed by progressive weakness and finally clonic convulsions. In the fatal cases the entire sequence of events usually occurred within five minutes after the injection.

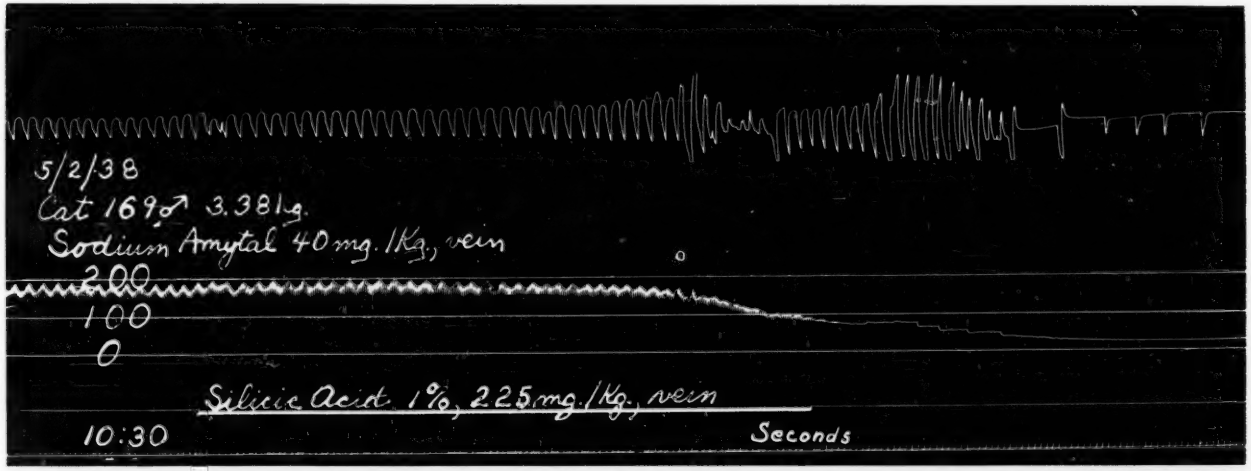


Fig. 1.—The effect of a massive intravenous dose of silicic acid on respiration, blood pressure, and heart rate. The cardiac pulsations toward the end of the tracing are too feeble to be visible in the reproduction.

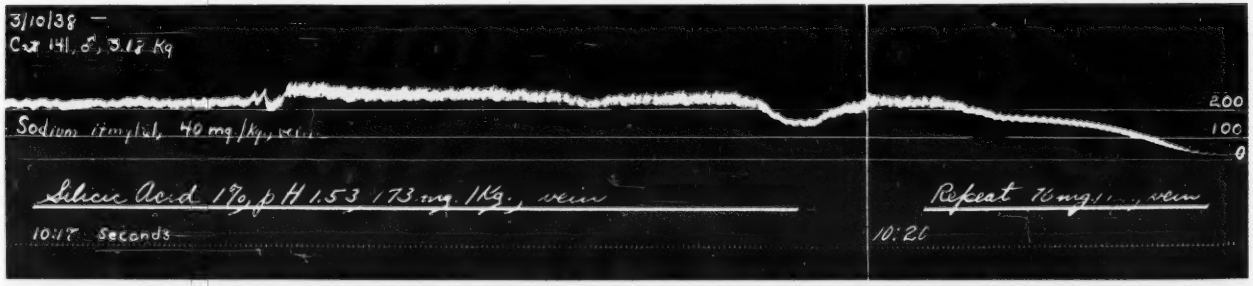


Fig. 2.—Complete recovery from an insufficient dose of silicic acid, with death after an additional dose.

In anesthetized cats records were made of the blood pressure, heart rate, and respiration (Fig. 1). In almost all cases there was an acceleration of respiration (from an average of 28 to an average of 48 per minute). In all cases there was a sharp fall in blood pressure (from an average of 180 to an average of 75 mm. Hg) and then a slow decline to zero. This was accompanied by slowing of the heart. In most cases respiratory stimulation occurred simultaneously with or soon after the fall in blood pressure. This was always followed by slowing, and finally cessation, of respiration. Cardiac pulsations persisted for several minutes after respiration had ceased. Convulsions were generally seen even in the presence of the anesthesia after the blood pressure had fallen.

A marked drop in blood pressure (about 75 to 100 mm. Hg) was rarely followed by recovery (Fig. 2). When the drop was less than 75 mm. and the injection was discontinued, recovery was usually prompt and often complete. However, in two instances after a drop there was a spontaneous but temporary recovery, and five and twenty-five minutes later, there was a second and fatal fall in blood pressure. This delayed effect was seen only twice during the entire study, although many experiments were conducted under similar conditions.

Records were also made of intrapulmonary blood pressure by cannulation of the left pulmonary artery (Fig. 3). When silicic acid was injected, the first effect seen was a rise in the systolic level of the intrapulmonary pressure which was followed by a rise in the diastolic level if more silicic acid was administered. These effects occurred before any change in systemic blood pressure was observed. It was only after there was a rise in intrapulmonic blood pressure that the systemic pressure began to fall. When the systemic pressure fell to about 50 mm. Hg, even under artificial respiration, diaphragmatic respiratory movements were accelerated and were soon followed by convulsions. When electrocardiograms were taken, they showed no change until after the blood pressure fell. The changes which then followed were of the type usually found in asphyxia.

An examination of the heart at the termination of all experiments in which silicic acid proved fatal revealed that the right side of the heart and the great vessels were distended with blood while the left side was essentially empty. Although the systemic blood pressure was at zero, the heart was usually contracting vigorously.

III. Mechanism: (a) Role of Blood Clot Formation.—It was noted by Gye and Purdy[2] that when silicic acid is administered intravenously, it produces massive intravascular clots. Since clots may produce systemic changes mechanically and mask the action of silicic acid, an anticoagulant dye, Chlorazol Fast Pink BKS,[3] was used to prevent the formation of clots. The dye produces no systemic effects in the amounts used.

Silicic acid was administered intravenously to two groups of six cats, only one group receiving Chlorazol. In both groups the symptoms preceding death, as well as the lethal dose, were the same. Examination after death in those treated with the Chlorazol revealed no clots.

(b) Site of Action.—The distention of the right side of the heart and the great veins, and the rise in intrapulmonic pressure after the intravenous administration of silicic acid may be explained by either failure of the right ven-

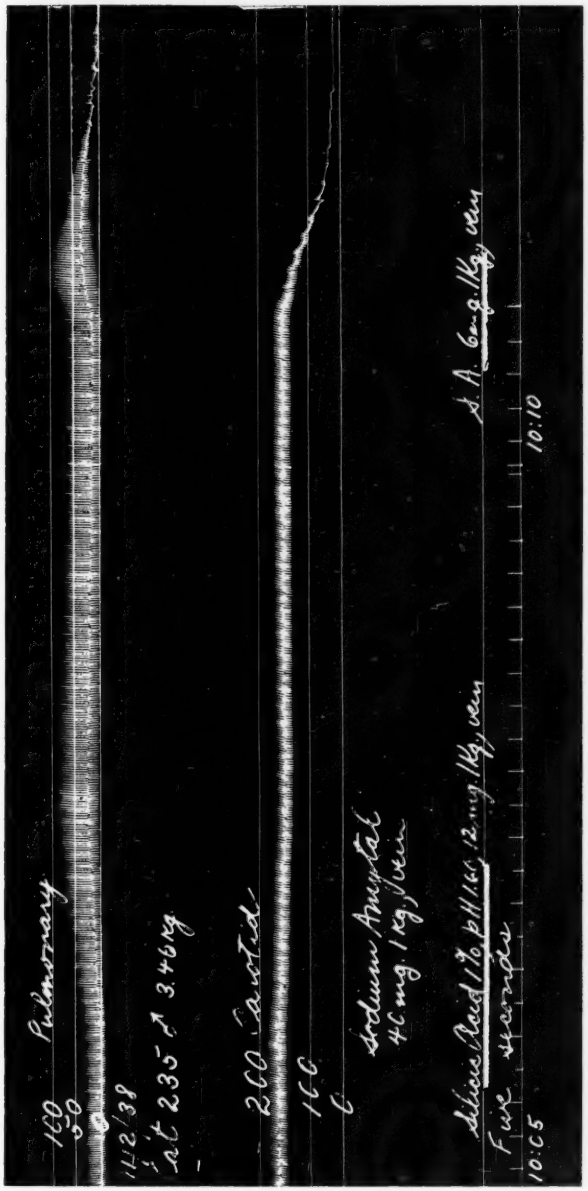


Fig. 3.—Effect of silicic acid on intrapulmonary blood pressure. The first dose raises the systolic level of intrapulmonary blood pressure and causes a slight drop in systemic blood pressure. The second dose causes a rise in the diastolic level of intrapulmonic blood pressure and a sharp fall in systemic blood pressure.

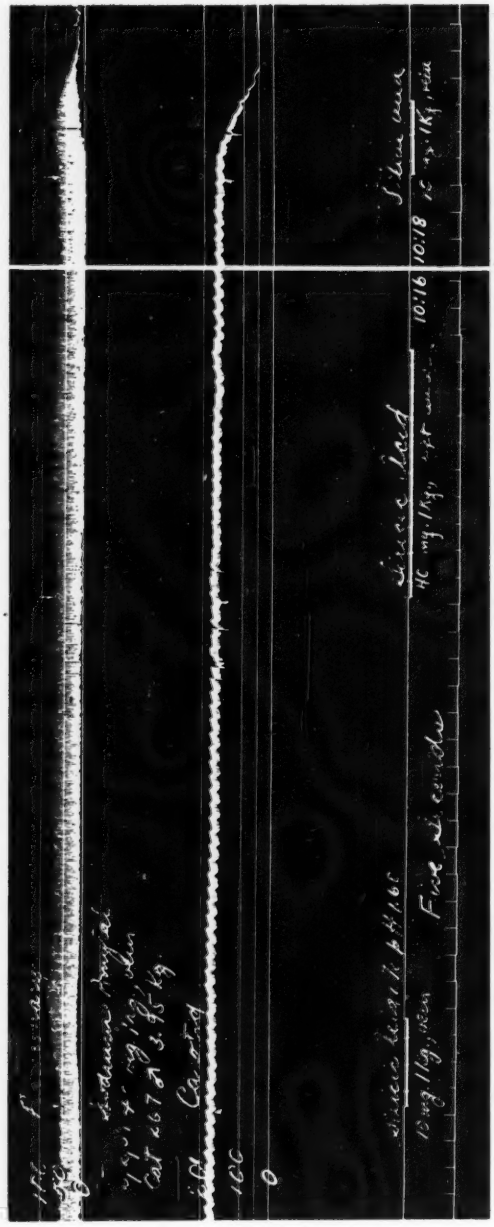


Fig. 4.—Effect of silicic acid when injected into the left auricle. The first dose administered intravenously produces a rise in both systolic and diastolic levels of intrapulmonic blood pressure, while a subsequent dose, four times as great, injected into the left auricle produces only an almost imperceptible rise in the systolic level of intrapulmonic blood pressure. The third dose injected intravenously, the same size as the first, is fatal.

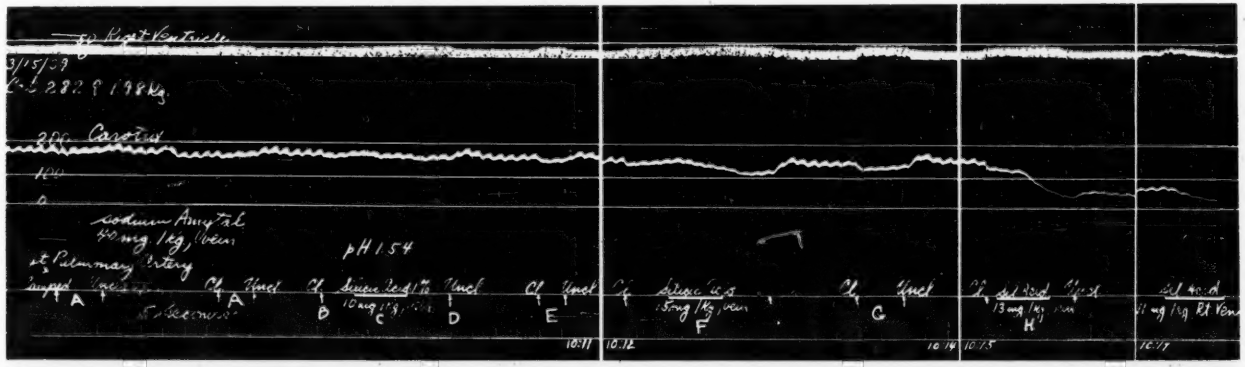


Fig. 5.—Effect of protection of lung from silicic acid. *Cl*, closing of left branch of pulmonary artery; *Uncl.*, opening of left branch of pulmonary artery; *A*, control showing effect of closing and opening of left branch of pulmonary artery before any silicic acid is injected. Closing produces very slight rise in pulmonic blood pressure and slight fall in systemic blood pressure. Opening restores to original level. *B*, closing of left pulmonary artery; *C*, injection of silicic acid with left lung out of circulation produces rise in pulmonic blood pressure and fall in systemic blood pressure; *D*, opening of left pulmonary artery. Blood pressure levels return to normal. *E*, closing of left pulmonary artery now produces greater rise in pulmonic blood pressure and fall in systemic blood pressure than in control (*A*); *F*, additional dose of silicic acid with left pulmonary artery closed produces more marked effect than before (as in *C*); *G*, now closing of pulmonary artery produces more pronounced effects on blood pressure than before, as in *A* and *E*; *H*, still more pronounced effects from another dose of silicic acid, with only slight recovery after opening of pulmonary artery.

tricle or block in the pulmonary circulation. The following observations were made in experiments designed to shed light on this question:

When silicic acid is injected intravenously, it reaches the right ventricle in relatively high concentration. It is conceivable, therefore, that the right ventricular muscle is damaged by the concentrated acid reaching it. Injections of silicic acid, therefore, were made into the left auricle, so that the acid reached the left ventricle before it circulated through the body. Such injections produced the same effects as the intravenous injections; namely, distention of the right side of the heart, rise in intrapulmonic blood pressure, and fall in systemic blood pressure. The only noticeable difference was that considerably more silicic acid was required to produce the same effects than was required with intravenous injections. Four times the dose which when injected intravenously produced marked changes in intrapulmonary pressure, was without effect when injected into the left auricle (Fig. 4).

It was also noted in a series of experiments that silicic acid was 25 per cent more toxic when the circulation of the left lung was ligated than when both lungs were intact. In another type of experiment (Fig. 5) the circulation of the left lung was constricted while silicic acid was being injected, and was re-opened after the injection had been completed. Thus the left lung was temporarily protected from the effects of silicic acid. The injection produced a rise in intrapulmonary pressure (recorded from the right ventricle) and a fall in systemic pressure. As soon as the protected lung was restored to the lesser circulation, both the intrapulmonary and systemic blood pressures tended toward normal. This protection against the effects of silicic acid was in evidence until massive (fatal) doses were given, when the protection afforded was only temporary. Control observations indicated that the constriction of the circulation to the left lung was itself without appreciable effect, whereas after the administration of silicic acid this procedure produced a definite rise in intrapulmonary and a fall in systemic blood pressures.

IV. Significance of the Colloidal Nature of Silicic Acid.—Inasmuch as silicic acid is a colloid and it has been demonstrated by Hanzlik[4] that colloids because of their physical nature can exert systemic effects, it was decided to determine whether the colloidal nature of silicic acid played any role in the effects observed.

Cats were injected with solutions of egg albumen, agar-agar, and acacia. Sufficiently large doses of these substances produced a fall in systemic pressure and an acceleration or respiration. However, in experiments with egg albumen and acacia, in which intrapulmonary blood pressure was determined, the initial effect was a rise in systemic pressure and not a rise in intrapulmonary pressure (Fig. 6).

Egg albumen does, however, exert a synergistic action on silicic acid. An initial dose of egg albumen (300 mg.), which produced only a transient fall in systemic blood pressure, considerably increased the toxicity of silicic acid administered two to three minutes later. In 8 cats the lethal dose of silicic acid averaged 165 mg., whereas after egg albumen the lethal dose of the same specimen for 7 cats averaged only 45 mg. In another series the control lethal dose of

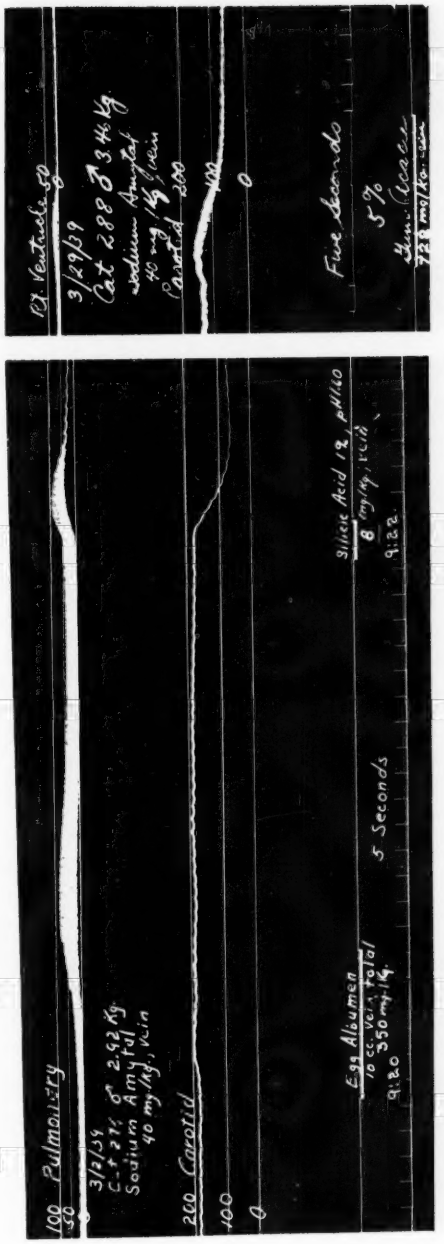


Fig. 6.—Effects of egg albumen and gum acacia on systemic blood pressure and pulmonary blood pressure.

silicic acid was 193 mg., and after egg albumen it was 110 mg. In both series the effects following the combination were the same as those following silicic acid alone.

DISCUSSION

The physical and chemical relationship between silicic acid and silica is very close; it has been claimed that silicic acid is hydrated silica,[5] and x-ray diffraction observations indicate that the atomic mosaic of both is very similar if not identical.[6] Gye and Purdy[2] have shown that silicic acid may, if administered over a long period of time, produce anatomical changes in organs other than the lungs, which are in many respects similar to those seen in the lungs in silicosis.

The lethal dose of silicic acid was very variable. There is no evidence that either the pH of the solutions used or the rate of administration of silicic acid played any role in the variable toxicity.

In the studies by Gye and Purdy it was demonstrated that death after intravenous injections of silicic acid was accompanied by massive intravascular clotting. These clots filled the chambers of the heart and many of the large veins. On the basis of other experiments these workers concluded that while clotting was the cause of death, it was secondary to endothelial damage. In our experiments it was demonstrated that the lethal dose and the toxic effects of silicic acid are not affected when the formation of clots is prevented by the use of an anticoagulant. It may be concluded, therefore, that while intravascular clotting may cause or contribute to the fatal outcome, there is another more direct action of silicic acid which may cause death.

The picture produced by ligation of the pulmonary artery, namely, distention of the right side of the heart, empty left side, rise in pulmonary blood pressure, and fall in systemic blood pressure, is exactly reproduced by the intravenous administration of silicic acid. The best explanation available for the production of the toxic effects of silicic acid is that it constricts the blood vessels of the lungs.

The question whether the effects of silicic acid are due to its chemical or physical nature is still an open one. Experiments by Hanzlik[4] indicate that colloids in general may produce marked changes in systemic blood pressure, but no colloid other than silicic acid has been demonstrated to produce an initial rise in intrapulmonary blood pressure. However, the synergism between silicic acid and egg albumen is suggestive of some common action.

SUMMARY AND CONCLUSIONS

Silicic acid solutions, prepared according to a standard procedure, differ markedly in their toxicity when injected intravenously into cats. The average lethal dose of different batches varied from 15 to 193 mg. per kilogram, and was not appreciably affected by wide range in pH or rate of injection.

Immediately following injection there was a rise in intrapulmonary arterial pressure and a fall in systemic blood pressure, with an accompanying engorgement of the right side of the heart and an empty left side. Similar changes followed when silicic acid first passed through the systemic capillary bed, as was the case when injection was made directly into the left auricle.

The effects on the lung circulation were not influenced by the use of an anticoagulant, and, therefore, they were not caused by intravascular clotting. Nor is it likely, in view of the selective action on the lung capillaries and the failure of

other colloidal substances (egg albumen, agar-agar, and acacia) to produce similar changes, that they represent a nonspecific colloidal effect of silicic acid.

It is concluded that silicic acid exerts a selective action on the capillary bed of the lungs which results in an increased resistance to blood flow and accounts for the toxic symptoms and death.

REFERENCES

1. Kettle, E. H.: Experimental Silicosis, J. Indust. Hyg. & Toxicol. **8**: 491, 1926.
Koppenhofer, G. F.: Untersuchungen zur Pathogenese silikotischer Gewebsveränderungen. IV. Mitteilung. Über die geweblichen Veränderungen nach experimenteller Zufuhr von kolloidaler Kieselsaüre, Virchows Arch. f. path. Anat. **297**: 271, 1936.
Hefferman, P.: Some Notes on the Biophysics of Silica and the Etiology of Silicosis, Brit. M. J. **2**: 489, 1929.
2. Gye, W. E., and Purdy, W. J.: Poisonous Properties of Colloidal Silica. I. Effects of the Parenteral Administration of Large Doses, Brit. J. Exper. Path. **3**: 75, 1922; Poisonous Properties of Colloidal Silica. II. Effects of Repeated Intravenous Injections on Rabbits; Fibrosis of the Liver, Ibid. **3**: 86, 1922; Poisonous Properties of Colloidal Silica. III. Ibid. **5**: 238, 1924.
3. Huggett, A. StG., and Rowe, F. M.: Azo-Dyes as Anti-Coagulants, J. Physiol., proc. **78**: 25, 1933.
Modell, W.: Chlorazol Fast Pink BKS as an Anti-Coagulant, Science **89**: 349, 1939.
4. Hanzlik, P. J., and Karsner, H. T.: Anaphylactoid Phenomena From the Intravenous Administration of Various Colloids, Arsenicals, and Other Agents, J. Pharmacol. & Exper. Therap. **14**: 379, 1920; A Comparison of the Prophylactic Effects of Atropine and Epinephrine in Anaphylactic Shock and Anaphylactoid Phenomena From Various Colloids and Arsphenamine, Ibid. **14**: 425, 1920; Further Observations on Anaphylactoid Phenomena From Various Agents Injected Intravenously, Ibid. **23**: 173, 1924.
Hanzlik, P. J.: Basis of Allergic Phenomena, J. A. M. A. **82**: 2001, 1924.
Hanzlik, P. J., De Eds, F., and Tainter, M. L.: Blood and Symptomatic Changes Following the Intravenous Administration of a Variety of Agents and Solutions, Arch. Int. Med. **36**: 447, 1925.
5. Weiser, H. B.: Inorganic Colloidal Chemistry, John Wiley & Sons, Inc., New York, 1935, Vol. 2, pages 199, 200, 226.
6. Krejci, L., and Ott, E.: Study of Silica Gel (X-ray Study), J. Physical. Chem. **35**: 2061, 1931.

TOXIC HEPATITIS DUE TO SULFANILAMIDE*

Report of a Fatal Case With Histopathologic Findings in the Liver

S. S. Berger, M.D., and H. S. Applebaum, M.D.
Cleveland, Ohio

THAT sulfanilamide has toxic effects is generally known. That it may cause hepatitis has been stressed by several observers. Hageman and Blake[1] have observed a case of toxic hepatitis, presumably due to sulfanilamide, in an alcoholic person. Saphirstein[2] recorded a severe but nonfatal case of hepatitis. Long[3] encountered jaundice with marked decrease in liver function on one occasion. Bannick, Brown, and Foster[4] reported two fatal cases of hepatitis in which they believed pre-existent liver damage a possibility. Garvin[5] recorded a series of five cases of hepatitis, one of which proved fatal, but no autopsy was obtained. Cline[6] was the first to place on record a case of yellow atrophy of the liver with autopsy findings. Fitzgibbon and Silver,[7] in a series of 200 cases showing toxic

*From the Medical Department, Mount Sinai Hospital, Cleveland.
Received for publication, December 16, 1939.

sulfanilamide effects, noted one severe nonfatal case of hepatitis. Fatal subacute atrophy of the liver with necropsy findings, however, is still sufficiently rare to warrant recording, this being only the second case to be reported in the English literature.

CASE REPORT

J. R., a white male, aged 67 years, was admitted to the surgical service of Mount Sinai Hospital on April 17, 1939, with the complaint of urinary frequency, hesitancy, and nocturia of five years' duration. He had had a similar attack ten years previous, but, following a cystoscopic examination, he seemingly improved for a period lasting five years. He had had pneumonia at the age of 12 years, and hay fever for the past four or five years. Physical examination revealed a few moist râles at both bases. His blood pressure was 180/90; examination of the abdomen gave negative findings; the prostate gland was hypertrophied, grade III. Laboratory data: phenolsulfonphthalein test was 70 per cent in three hours; blood chemistry was normal; Kline tests were negative; urine: albumin two plus; sugar (negative); blood count: innumerable red blood cells; hemoglobin 90 per cent, white blood cells 7,500. As part of preoperative treatment the patient was given on April 18, 10 grains of sulfanilamide three times a day for three days (total 100 grains) without any apparent untoward symptoms. Suprapubic prostatectomy was performed on April 22 under spinal anesthesia. Pathologic diagnosis: chronic and subacute prostatitis with nodular glandular hypertrophy and considerable hyperplasia. The patient made a good recovery and was discharged on May 10.

He was readmitted to the medical department on June 16, 1939. He stated that he felt well after leaving the hospital until about June 2, when he was given by his attending physician six 5-grain tablets of sulfanilamide daily for ten days (total of 20 Gm.) on account of pyuria. Soon after starting the drug he began to have anorexia, nausea, weakness, and began to pass "coffee-like" urine and light-colored stools. The patient blamed the medicine for his symptoms and stated that as soon as the drug was discontinued these symptoms subsided. Physical examination on this admission showed a considerable degree of jaundice, an enlarged liver palpable two fingerbreadths below the costal margin, and the edge of the spleen was palpable. There was no ascites, and there were no visible distended veins. The blood pressure was 136/84. Impression: toxic hepatitis probably due to sulfanilamide. (Dr. G., the family physician, reported that jaundice was not present on June 12, three days before admission.) Course: the jaundice increased rapidly. There was no urobilin or urobilinogen in the urine, and the stool was negative to hydrobilirubin except on July 10 and 15, when it was slightly positive. The jaundice was of the obstructive type, and it was our opinion, chiefly because of the enlarged spleen, that the patient had a toxic hepatitis merging into a chronic state, due to sulfanilamide. On July 12 signs of ascites, which progressively increased, were in evidence. On July 19 the patient was released at his own request. Laboratory examination during this admission was as follows: gastrointestinal series was negative; flat plate of the right upper quadrant was negative; electrocardiogram: P-R interval twenty-three seconds and slight slurring of QRS wave in limb leads; urine: bilirubin was positive in all specimens; urobilinogen negative; clumps of white blood cells; no leucine or tyrosine; blood: mild normocytic anemia, hemoglobin 75 to 76 per cent; white blood cells 5,700, red blood cells 3.8 million; icteric index varied between 90-67-115; van den Bergh test 19.2 units on June 17; cholesterol 210-203; esters 50 to 30; chemistry: nonprotein nitrogen 30, creatine 1.5; total plasma proteins 5.70; albumin 4.45; globulin 1.25; Kline test: diagnostic two plus, exclusion four plus on June 17 and June 19 (however, negative on first and on last admissions); stool hydrobilirubin was negative (eighteen examinations) except on July 10 and July 15, when it was slightly positive; ascitic fluid: no fixed tissue present; no cells. Pathologic report: fibrin clot. Treatment consisted of high carbohydrate diet supplemented by glucose and high vitamin diet and some bile salts, and paracentesis when indicated. Weight on admission was 160 pounds; on discharge, 163 pounds.

The patient was admitted to another service for paracentesis and peritoneoscopy on July 28. His diagnosis was cirrhosis of the liver.

The patient's fourth admission to the hospital and his second admission to the medical service occurred on August 4. At that time jaundice was intense, and ascites had increased. The liver edge was not felt and percussion showed it to be smaller than on the previous admission; the abdomen was distended with fluid. The course now was progressively and rapidly downward. On August 14 the patient began to get drowsy (cholemia); this drowsiness gradually merged into coma and he died on August 22, 1939.

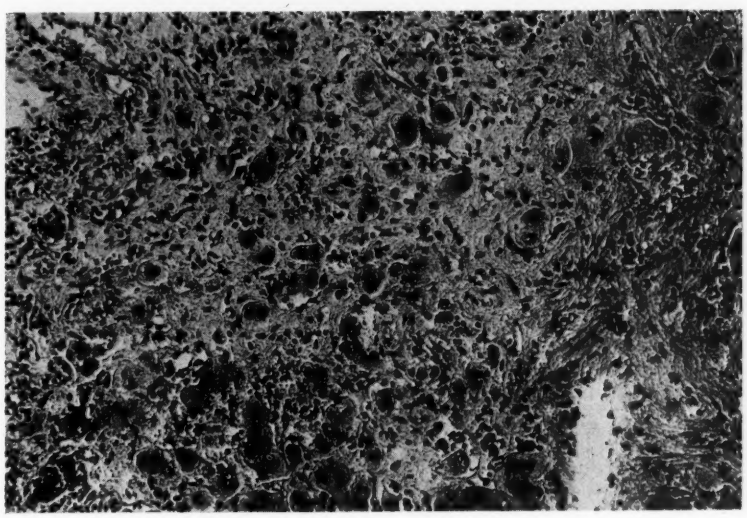


Fig. 1.—Liver. Low-power magnification. Extensive necrosis with replacement fibrosis. ("Toxic hepatitis." "Subacute yellow atrophy.")

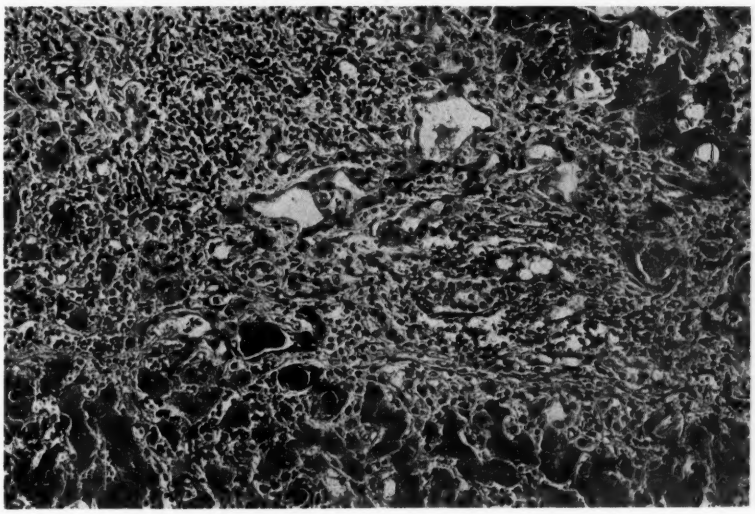


Fig. 2.—Liver. Low-power magnification. Extensive necrosis with replacement fibrosis. Beginning cirrhosis. ("Toxic cirrhosis.")

On this admission nonprotein nitrogen was 34, icteric index was 120, cholesterol 180, esters 27.5, total plasma proteins 4.65, albumin 2.32 and globulin 2.33. In the urine bilirubin was positive; urobilinogen was positive (hydrobilirubin, however, was negative on three examinations); leucine and tyrosine were negative. Blood count showed red blood cells 4 million, hemoglobin 75 per cent, white blood cells 8,000 to 12,000. Bleeding time was two minutes and clotting time was four minutes. Kline test was negative. Abdominal fluid was tapped and showed abundant growth of *Staphylococcus aureus* (contamination). The temperature

was normal throughout his hospital stay, except on admission (101.5 on June 16), on June 27 (100.5), and for a week following operation. Final diagnosis was subacute yellow atrophy of the liver and cholemia.

Permission for an autopsy was not obtained, but through a puncture wound we were able to obtain several small segments of liver tissue which form the basis of the pathologic report submitted by our pathologists, Drs. B. S. Kline and A. M. Young, as follows:

Autopsy was limited. The liver is small, firm, and finely granular. On microscopic examination the liver cells in many places show early degenerative changes with some of the nuclei pyknotic and deeply blue stained, and others poorly stained or unstained with cell outlines blurred. In these areas there is abundant granular greenish-yellow bile pigment in the cytoplasm of the liver cells and homogeneous bile in the canaliculi. In other areas the liver cells are necrotic (see Fig. 1) and in many places they are absent, with apparent condensation of the stroma and bile ducts. Many of the small bile ducts are overdistended by homogeneous masses of bile. There is considerable infiltration in the periportal areas by wandering cells, with small round cells predominating (see Fig. 2). The normal lobulation of the liver is destroyed, the intact liver tissue occurring in irregular small rounded nodules, characteristic of beginning cirrhosis. Anatomic diagnosis: extensive necrosis and replacement fibrosis, liver (''toxic hepatitis,'' ''subacute yellow atrophy,'' and beginning cirrhosis, ''toxic cirrhosis'').

The patient apparently had a normal liver by the usual standards of examination, until he began to take sulfanilamide. No other drug was taken, and there is no history of previous contact with any hepatic irritants. He ingested 6.6 Gm. of sulfanilamide over a period of three days, April 18 to April 22, and again a second course of 20 Gm. over a period of ten days, June 2 to June 12. This was promptly followed by gastric upset, with nausea and vomiting, and about four days after the discontinuance of the drug he developed jaundice, enlarged liver and spleen, and later ascites. It is reasonable, if not certain, to accept a causal relationship between the drug and the hepatitis. Further, presumptive evidence may be had in the patient's own statement that gastric disturbance promptly followed the taking of the tablets, and promptly disappeared upon discontinuance of them. This case differs from the two fatal cases of hepatitis reported by Bannick, Brown, and Foster[4] in that they believed that their patients might possibly have suffered from pre-existing liver damage; it differs also from Garvin's[5] fatal case, in which hepatitis was complicated by exfoliative dermatitis and anemia. There were no skin lesions and no appreciable anemia in our case, and, as stated before, no evidence of previous liver damage. The Kline test was completely negative at first, then positive (diagnostic two plus, exclusion four plus), and again completely negative, and there was nothing in the history or physical examination to support the suspicion of a syphilitic involvement. Because of the age of the patient (67) and the painless progressive jaundice of the obstructive type, the possibility of malignancy suggested itself. However, the enlarged spleen, which was readily palpable on the first admission, made the differential diagnosis in favor of hepatitis and cirrhosis, and finally subacute atrophy (necrosis) of the liver. This impression was supported by a definite history of sulfanilamide ingestion, the absence of malignant cells from the ascitic fluid, the negative peritoneoscopic examination as regards tumors, the interruption in

the complete obstruction, as evidenced by the occasional finding of hydrobilirubin in the stool and urobilinogen in the urine, plus the subsequent decrease in size of the liver.

It is of interest to note that Saphirstein's[2] patient had an associated exfoliative dermatitis, and so did three of Garvin's[5] series of five patients, and that the only fatality was one of these patients. Our own patient was apparently free from all skin lesions during his present illness; so was the one of Cline[6] and of Fitzgibbon and Silver.[7]

This, then, is the second case of sulfanilamide hepatitis with post-mortem findings of the liver to be reported. With the increasing use of this drug more cases of hepatitis are to be expected. Whether this injury is due to hypersensitivity or to inherent toxicity, it is difficult to say. The case of Fitzgibbon and Silver[7] suggests the former. After an interval of six weeks following the last dose of sulfanilamide, their patient took 15 grains (1 Gm.) more of this drug and six days later developed severe hepatitis. Similarly, in our case there was an interval of about six weeks between the two courses of the drug, and the appearance of jaundice occurred within four days after its discontinuance. Also, in 7 of 32 patients of Bannick and associates[4] the previous administration of sulfanilamide rendered further treatment with this agent inadvisable. Recently, one of us (H. S. A.) had a similar experience with sulfapyridine. Repeating the drug after an interval of seven days brought on such severe gastric disturbance that it necessitated prompt discontinuance of the sulfapyridine, bringing prompt relief of the symptoms. Some of the patients may eventually overcome these toxic effects with the continuance of the drug, but it is a much safer procedure to discontinue it.

On the other hand, Hageman and Blake's[1] researches failed to demonstrate any precipitins for sulfanilamide in the blood serum during and following the reaction, as have attempts to demonstrate sensitivity by the patch and intradermal tests. Passive sensitization in human beings and in guinea pigs have also yielded negative results. Attempts to produce anaphylactic and skin hypersensitivity in guinea pigs have likewise failed. The prevailing view (Silver and Elliott,[10] Garvin,[5] and others) at present is that the sensitization phenomena are similar to those which occur with arsphenamine, cinchophen, and aminopyrine.

There was a history of hay fever in our patient of about four to five years' duration. However, there was no opportunity for investigation of this angle, and no further enlightenment could be obtained from the family physician on this point. Its presence would have raised more than mere speculation as to the possibility of an allergic basis for the hepatitis following the second course of sulfanilamide therapy.

Apparently the dosage of sulfanilamide is not the only factor in its toxicity. Clark[9] reports a patient who had taken 290 grains (18.85 Gm.) of sulfanilamide orally at one time "with no more serious effects than a transient decrease in the respiratory rate and a marked drowsiness, both of which symptoms were entirely relieved within twenty-four hours by forced fluids and respiratory stimulants." Cline's patient[6] who died received 45 Gm. of sulfanil-

amide and the one of Fitzgibbon and Silver[7] 500 grains (33.3 Gm.) of sulfanilamide. Our patient received the smallest amount, a total of 26.6 Gm. of the drug. While there is an accepted general plan of dosage, each case is an individual problem as to how much sulfanilamide it should receive. Long's[8] studies indicate the value of a concentration of 10 to 15 mg. of the drug per 100 c.c. of blood in the fulminating cases, and from 5 to 10 mg. per 100 c.c. of the blood as a favorable concentration to obtain good results in the mild and moderate cases.

SUMMARY

We have presented a case of fatal hepatitis (subacute yellow atrophy of the liver) following the ingestion of 26.6 Gm. of sulfanilamide, and have presented the post-mortem findings of sections of the liver. This experience confirms the view of Garvin and others that sulfanilamide should be added to the list of agents which may cause severe damage to the liver, and further emphasizes the fact that it may even prove fatal. The danger of the indiscriminate use of sulfanilamide thus becomes obvious.

REFERENCES

1. Hageman, P. O., and Blake, F. G.: A Specific Febrile Reaction to Sulfanilamide: Drug Fever, J. A. M. A. **109**: 642, 1937.
2. Saphirstein, H.: Hepatitis and Toxic Erythema With Desquamation Due to Sulfanilamide, Urol. & Cutan. Rev. **42**: 101, 1938.
3. Long, P. H.: The Clinical Use of Sulfanilamide and Its Derivatives With Special Reference to Their Possible Toxic Effects, Ohio State M. J. **34**: 977, 1938.
4. Bannick, E. G., Brown, A. E., and Foster, F. P.: Therapeutic Effectiveness and Toxicity of Sulfanilamide and Several Related Compounds, J. A. M. A. **111**: 770, 1938.
5. Garvin, Curtis F.: Toxic Hepatitis Due to Sulfanilamide, J. A. M. A. **111**: 2283, 1938.
6. Cline, E. W.: Acute Yellow Atrophy of the Liver Following Sulfanilamide Medication, J. A. M. A. **111**: 2384, 1938.
7. Fitzgibbon, P., and Silver, B.: Necrosis of Liver (Case), California & West. Med. **50**: 123, 1939.
8. Long, P. H., Bliss, E. A., and Feinstone, W. H.: Mode of Action and Clinical Use and Toxic Manifestations of Sulfanilamide, J. A. M. A. **112**: 115, 1939.
9. Clark, A. L.: Sulfanilamide in Treatment of Nonspecific Infections of the Urinary Tract, J. A. M. A. **112**: 719, 1939.
10. Silver, B., and Elliott, M.: The Use of Sulfanilamide in 1625 Cases of Gonorrhea in the Male, J. A. M. A. **112**: 723, 1939.

TRANSIENT POSITIVE SEROLOGIC TESTS FOR SYPHILIS*

ROBERT A. GREENE, PH.D., AND EDWARD L. BREAZEALE, B.S.A.
TUCSON, ARIZ.

IT IS generally recognized that syphilitic sera, upon storage, may gradually decrease in potency. As a general rule, these changes are fairly slow. It is common practice among smaller laboratories to hold positive sera for several weeks for use as controls in the performance of serologic tests.

In connection with an exchange of sera with other laboratories, we frequently noted that some which gave positive reactions in one laboratory gave negative reactions in others within a relatively short time interval. In other cases, the potency of sera remained practically unchanged over a period of time. Since the changes occurred chiefly in weakly positive sera, we attributed these changes to a decrease in reagin content. In weakly positive sera variations in sensitivity of antigens would be most pronounced.

The following event called our attention to the fact that changes may be even more pronounced. A young Mexican girl had applied for admission to a hospital for tubercular children. A blood specimen gave positive Kline, Hinton, and Kahn reactions. A second specimen gave negative reactions. A third specimen, which was divided, gave positive Kline, Hinton, and Kahn reactions in this laboratory, and negative Kahn and Wassermann reactions in another laboratory. The serum remaining from our tests was immediately referred to two other laboratories; one reported a negative Kahn reaction, the other negative Kline and Kahn reactions. The serum was returned to us, and upon re-examination gave negative Kline, Kahn, and Hinton reactions.

A fourth specimen gave negative reactions. A fifth specimen was examined separately so as to eliminate any possibility of mixing with other specimens. The specimen gave a positive Hinton, and strongly positive Kahn and Kline tests. The negative and positive controls gave negative and positive reactions, respectively. The serum and the controls were referred to two other laboratories; one reported a negative Kahn reaction, the other negative Kline and Kahn reactions. Both reported the negative control as negative and the positive control as positive. The serum was returned to this laboratory and re-examined. The serum gave negative Kline, Hinton, and Kahn reactions; the negative and positive controls gave negative and positive reactions, respectively.

The total time elapsing between our first and second examinations was less than three hours. The same lots of Kline antigen emulsion and Hinton glycerinated indicator were employed. The following day the serum was heated for ten minutes at 56° C. water bath and re-examined. The Kline, Kahn, and Hinton reactions were negative.

*From the Arizona State Laboratory, Tucson.
Received for publication, December 18, 1939.

As a result of this experience we decided to re-examine a number of sera on the day following examination. Our routine procedure has been to separate the sera, inactivate them for thirty minutes at 56° C. in an electrically controlled water bath, allow them to cool to room temperature, and then examine them immediately. When sera had been stored in the icebox, they were reheated for ten minutes in a 56° C. water bath before examination. In every case, the same lots of Hinton indicator and Kline antigen emulsion were used.

RESULTS

Several hundred sera have been examined in the manner just outlined. A large number of weakly reacting sera were negative upon recheck; as a general rule, strongly positive sera retained their potency over rather long periods of time. To date, we have not encountered a serum which gave negative reactions on the first test and positive reactions upon recheck. The results in Table I are representative of a large number of similar observations.

TABLE I

SPECIMEN NUMBER	EXAMINED IMMEDIATELY AFTER INACTIVATION			EXAMINED LATER			TIME BETWEEN TESTS HOURS
	KLINE	HINTON	KAHN	KLINE	HINTON	KAHN	
19236	2+	--	--	--	--	--	4
19245	3+	--	--	1+	--	--	4
19254	3+	--	--	--	--	--	24
19641	4+	+	4+	1+	+	1+	48
20180	3+	+	4+	±	±	±	24
20199	2+	+	2+	±	±	±	24
20522	2+	+	2+	--	--	--	24
20642	4+	+	4+	1+	±	1+	24
21143	2+	+	3+	2+		±	24
23610	2+	+	2+	--	--	--	24
25396	2+	+	1+	--	--	--	24
25441	3+	+	3+	2+		1+	24
26750	4+	+	4+	--	--	--	4
27890	2+	+	2+	--	--	--	4
29000	4+	+	4+	--	--	--	4

DISCUSSION

Although most of the sera which rapidly dropped in titer gave weakly positive reactions, a much larger number of weakly reacting sera continued to give weak reactions over a period of time. On the other hand, the occurrence of strongly positive reactions, which become negative within a few hours, presents a problem. At first, we felt that this might be a method of eliminating "nonspecific" reactions. During this study we have found, however, that in some cases, sera from known syphilities might change from positive to negative, and reacting sera from individuals who were proved to be nonsyphilitic might retain their potency; in other cases, the situation might be reversed.

The simplest explanation would be that of poor performance of the laboratory tests. However, our branch laboratory at Phoenix made a very satisfactory record in the 1939 Evaluation Study, and an interlaboratory check over a long period has shown that the tests performed in the Tucson laboratory fall well within recommended limits of sensitivity and specificity.

The possibility of daily variation in sensitivity of the tests does not seem to be an adequate explanation. The same suspensions of Kline antigen emulsion and Hinton glycerinated indicator used in the first test were employed in subsequent examinations of the same serum. In a single series many specimens which gave weakly or strongly positive reactions on the first test, gave identical reactions upon re-examination at a later date. In no case did a negative serum give a positive reaction upon recheck.

SUMMARY

It was found that certain sera which gave positive Kline, Hinton, and Kahn tests, gave negative reactions when re-examined in a relatively short period of time.

AN EVALUATION OF THE COMBINED EFFECTS OF SULFAPYRIDINE AND THE BARBITURATES IN THE TREATMENT OF PNEUMONIA*

WILLIAM L. M. KING, M.D., AND HERMAN J. MOERSCH, M.D.
ROCHESTER, MINN.

BECAUSE of a report by Adriani[1] of New York in July, 1939, to the effect that rats placed under treatment with sulfanilamide were thereby rendered highly susceptible to the action of the barbiturates, we undertook this study. Adriani demonstrated that in rats so treated and then administered the sodium salts of methyl-butyl-ethyl-thiobarbituric acid (pentothal), isoamyl-ethyl-thiobarbiturate (thio-ethamyl), N-methyl-cyclo-hexenyl-methyl-barbituric acid (evipal), isoamyl-ethyl-barbituric acid (amytal), or methyl-butyl-ethyl-barbituric acid (nembutal), subanesthetic doses of these agents became anesthetic, and anesthetic doses of the same agents usually became lethal. The various anesthetic inhalants, such as ether and cyclopropane, on the other hand, did not have this effect on the sulfanilamide-treated rats, nor did tribrom-ethanol. The dose of sulfanilamide given the rats was from 0.5 to 1.0 mg. per gram of body weight daily for three days. Adriani concluded his report by suggesting that the combination of sulfanilamide and barbiturates may be unwise in human therapy.

We have reviewed 18 cases of primary pneumonia to determine whether the customary use of barbiturates as sedatives in conjunction with sulfapyridine had produced any recognizable deleterious effects. In one case the clinical diagnosis was pneumonia and the Neufeld reaction was positive, but the results of two roentgenologic examinations of the thorax were negative. This case was included because both sulfapyridine and pentobarbital sodium had been used during treatment of the patient.

We have presented in Table I the respective total doses of sulfapyridine or sulfanilamide and the particular barbiturate used in each case, together with the patient's age, the result of typing his sputum, and a short comment on the

*From the Mayo Clinic, Rochester.
Received for publication, December 26, 1939.

TABLE I

TOTAL DOSES OF ANTISEPTIC DRUGS AND BARBITURATES ADMINISTERED

PATIENT'S AGE	NEUFELD REACTION	DOSE OF ANTISEPTIC DRUG				DOSE OF BARBITURATE										REMARKS
		SULFAPYRIDINE		SULFANILAMIDE		PENTOBARBITAL SODIUM		SODIUM AMYTAL		SECONAL		PHENOBARBITAL SODIUM		PHENOBARBITAL		
		GR.	GM.	GR.	GM.	GR.	GM.	GR.	GM.	GR.	GM.	GR.	GM.	GR.	GM.	
3 mo.	Positive	16	1.0			0.75	0.05									No ill effects from drugs; uneventful recovery
10 mo.	Negative	109.5	7.1			8.25	0.53									No ill effects from drugs; uneventful recovery
11 mo.	Negative	10.5	0.7											0.5	0.032	No ill effects from drugs; uneventful recovery
19 mo.	Positive			158	10.2	9.75	0.63									No ill effects from drugs; prolonged illness
3½ yr.	Negative	75	4.9											0.25	0.016	No ill effects from drugs; uneventful recovery
5 yr.	Positive	240	15.6			1.5	0.1									No ill effects from drugs; recurrence of fever
6 yr.	Negative	90	5.0			0.75	0.05									No ill effects from drugs; uneventful recovery
11 yr.	Negative	370	24.0			10.75	0.70									No ill effects; long illness; left pleural effusion
16 yr.	Positive	460	29.8			3	0.2									No ill effects from drugs; x-ray of chest negative
18 yr.	Negative	585	37.9			8.25	0.53			3	0.2	10	0.65			No ill effects; long illness; right pleural effusion
26 yr.	Negative	675	43.7							4.5	0.3					No ill effects; long illness; complicating parotitis
27 yr.*	Positive	380	24.6					9	0.6							Stuporous for 18 hours after receiving sodium amytal, gr. 6 (0.4 Gm.)
30 yr.	Positive	60	3.9			1.5	0.1									No ill effects from drugs; uneventful recovery
34 yr.	Negative	225	14.6			3	0.2	3	0.2					1.5	0.1	No ill effects from drugs; uneventful recovery
37 yr.	Negative	245	15.9			7.5	0.5									No ill effects from drugs; uneventful recovery
45 yr.	Negative	150	9.7							3	0.2					No ill effects; long illness; left pleural effusion
70 yr.	Negative	255	16.5	160	10.4			6	0.4							No ill effects; died of hypertensive cardiac failure
92 yr.	Positive	240	15.6			3	0.2	3	0.2							Much nausea; stormy course; recurrence of fever

*A detailed description of this case will be found in the text.

course of his illness and the complications, if any. It will be seen that the most frequently used barbiturate was pentobarbital sodium and that the dose of sulfapyridine in the adult patients averaged approximately 330 grains (21.4 Gm.). The ages of the patients ranged from 3 months to 92 years. The Neufeld reaction was positive for a specific type of pneumococcus in 7 of the 18 cases. In a careful analysis of the hospital records, we were unable to find any suggestion that an untoward reaction that might be attributed to a combined effect of the two types of drugs had occurred in any of the cases, save one. This was true irrespective of the age of the patient, the type of pneumonia from which he was suffering, or the presence of complicating factors, such as pleural effusion or parotitis.

The one exception involved was a woman aged 27 years, who was in her seventh month of pregnancy and who had a lobar type of pneumonia involving the left lower lobe caused by pneumococcus type XII. The day after her admission to the hospital, her temperature decreased from 103.6° F. (39.8°C.) to normal, but on the third day she began to go into labor. Six grains (0.4 Gm.) of sodium amytal and $\frac{1}{200}$ grain (0.00032 Gm.) of hyoscine were administered and she went into a deep sleep from which she could not be roused for eighteen hours. The total dosage of sulfapyridine administered up to that time was 225 grains (approximately 14.6 Gm.). The onset of labor was delayed thirteen days.

SUMMARY AND CONCLUSIONS

We have studied 18 cases in which primary lobar pneumonia or bronchopneumonia has been treated with sulfapyridine or sulfanilamide and in which the patient was given one or more of the barbiturates in sedative doses. No untoward effects were observed, except in one instance in which prolonged stupor of the patient followed the administration of 6 grains (0.4 Gm.) of sodium amytal following a previous dosage of 225 grains (approximately 14.6 Gm.) of sulfapyridine. That this reaction was the result of the combined effect of the two drugs can be postulated, but the postulation would be difficult to prove.

It may be concluded, therefore, that the barbiturates in ordinary sedative doses may be used with apparent immunity for patients receiving sulfapyridine or sulfanilamide.

REFERENCE

1. Adriani, John: Effects of Anesthetic Drugs Upon Rats Treated With Sulfanilamide, J. Lab. & Clin. Med. 24: 1066, 1939.

CLEARANCE TESTS IN RELATION TO RENAL PATHOLOGY*

ESTHER M. GREISHEIMER, ROBERTA HAFKESBRING, AND HULDA MAGALHAES
PHILADELPHIA, PA.

FOR some time we have been studying the effects of various anesthetics on kidney function. In this report the results of 275 urea clearance tests and 106 creatinine clearance tests on 10 dogs are presented. The following range of control values of urea clearance per square meter per minute has been noted: Orth,[1] 34.9 c.c.; Rhoads,[2] 49.8; and Ralli,[3] 99.5. Because of this great variation and the inconstant results in some of our dogs under anesthesia, it seemed advisable to do autopsies at the end of our study. We are deeply indebted to Professor Helen Ingleby and her staff for autopsy reports on 7 of our dogs. Although complete autopsies were performed, only the kidney findings will be included in this paper.

On each experimental day, body weight, blood pressure (Allen[4]), respiratory rate, heart rate, rectal temperature, erythrocyte and leucocyte counts, and hemoglobin (Newcomer method) were determined. The average control values for each dog are shown in Table I. Urea clearances were done by the Van Slyke[2] hypobromite[5] method; creatinine clearances were done by the Holten and Rehberg[6] method. Adult female dogs, in reasonably good health and well trained, were used. They were allowed to lie comfortably between catheterizations on the laboratory floor on blankets. Tests were done eighteen to twenty-one hours after the last meal, and no more than two tests were done on one dog in one week. The dogs were permitted to exercise daily on the roof. Some of the dogs were studied for as long as sixteen months before coming to autopsy.

Some of the dogs were fed Red Heart C, some Victory Dog Food, and the remainder Cero-Meato. Three dogs were studied for several months on each of the diets in turn, and no significant changes in urea clearance were found. We noticed that all the dogs gained weight on Cero-Meato; the pathologist stated that the dogs which had been fed Cero-Meato seemed to be in better general condition at the time of autopsy than the dogs which had been fed either of the other diets.

A brief report of the results of each anesthetic will be given. In each anesthetic period surgical anesthesia was maintained for one hour. The clearance tests on the day after the anesthesia were compared with those before and with those one, two, and more weeks after.

*From the Department of Physiology, Woman's Medical College of Pennsylvania, Philadelphia.

This study was made possible by grants from the Penrose Fund of the American Philosophical Society and the Committee on Therapeutic Research, Council of Pharmacy and Chemistry, American Medical Association.

Received for publication, December 26, 1939.

TABLE I

1. Number of tests
2. Diet
3. Rectal temperature
4. Respiratory rate
5. Blood pressure
6. Heart rate
7. Blood urea nitrogen
8. Blood creatinine
9. Urea clearance
10. Range—urea clearance
11. Creatinine clearance
12. Hemoglobin (Gm.)
13. R.B.C. count (millions)
14. W.B.C. count (thousands)

AVERAGE OF CONTROL VALUES DURING STANDARDIZATION

DOG	R	N	W	C	D	K	L	M	F	B
1	4	4	12	5	4	5	4	6	4	8
2	Red Heart C	Red Heart C	Red Heart C	Victory	Victory	Cero-Meato	Cero-Meato	Cero-Meato	Victory	Cero-Meato
3	100.4	100.8	101.7	100.7	100.9	100.7	102.2	100.4	101.4	101.4
4	17	24	45	25	28	26	18	17	26	18
5	162/72	220/111	164/83	160/60	168/69	167/60	161/56	167/60	157/67	166/60
6	136*	116*	114*	86	82	89	96	85	83	95
7	12.0	17.2	12.3	11.2	11.7	14.6	12.0	14.4	9.3	11.9
8	—	—	—	—	—	9.64	8.09	10.35	—	—
9	56.2	54.9	55.5	61.0	40.5	64.3	71.3	59.5	59.9	64.5
10	52.9-57.8	52.0-58.8	47.5-69.9	53.8-67.4	32.8-44.8	54.2-88.0	51.1-110.9	55.3-68.5	56.8-64.4	35.7-132.3
11	—	—	—	—	—	75.8	74.3	77.1	—	—
12	11.57	13.41	12.21	10.92	11.88	10.60	11.12	11.10	10.34	11.00
13	5.75	6.63	6.62	6.07	7.41	7.28	7.38	7.82	5.46	6.35
14	5.4	8.6	10.0	12.1	9.8	17.0	10.2	14.6	9.2	12.0

*The heart rate was determined from the electrocardiogram instead of by stethoscope.

(a) Cyclopropane* (Diet—Red Heart C)

1. Dog R. During eight months cyclopropane was administered ten times, and the dog seemed in excellent health throughout. The most significant finding was the increase of about 40 per cent in the urea clearance on the day after the anesthesia; this was noted each time cyclopropane was administered. The average control value was 56.2 c.c. per square meter per minute, as shown in Table I, and the average value for the day after anesthesia was 79.5; this finding does not agree with that of Orth.[1] The urea clearance returned to the control value within one week after the anesthesia.

After 42 clearance tests and ten administrations of cyclopropane this dog was brought to autopsy by a lethal dose of the same anesthetic. The kidneys showed congestion only; consequently, we feel that these results are typical of cyclopropane.

2. Dog N. It may be noted in Table I that this dog showed a higher blood urea nitrogen than any of the others, and it was the only one with hypertension. It showed a rather fixed urea clearance in the 11 tests performed. Cyclopropane was administered only three times, since the dog died just at the end of the third period. The control value for urea clearance was 54.9; after the anesthesia it was 54.3.

At autopsy the kidneys showed capillary engorgement of the glomeruli and cloudy swelling of the tubules. This pathology, together with the hypertension and higher blood urea nitrogen, probably was a determining factor in the fixed clearance.

3. Dog W. Thirteen clearance tests were done on this dog. The average control value was 55.5; after one administration of cyclopropane it increased

*We are greatly indebted to the Foregger Company for the generous loan of the Foregger instrument, Metric Model No. 4, for administration of cyclopropane.

to 66.7, or about 20 per cent. Since this dog was not able to be trained to our satisfaction, it was returned to the kennels.

(b) Pentobarbital sodium (Abbott) (Diet—Victory Dog Food)

Twenty-two anesthetic periods were studied on Dogs C, D, and F. The following results were found for urea clearance[7]:

DOG	CONTROL	AFTER ANESTHESIA
C	61.0	55.2
D	40.5	44.7
F	59.9	69.0

Since comparable results were found at some time during the establishing of the control values in two of the dogs, we felt that there were no significant changes which could be attributed to the anesthesia.

(c) Sedative dose of pentobarbital sodium followed by ether, drop method, for one hour (Diet—Red Heart C)

Dogs C, D, and F were left in the kennels for the summer and were studied again in the autumn. After new controls were established, each dog was given ether once. The following results were found:

DOG	NEW CONTROL	AFTER ANESTHESIA
C	56.7	72.2
D	47.0	49.9
F	64.8	86.7

The urea clearance increased markedly in two and only slightly in one the day after ether was administered. Orth[1] reported practically no change after ether. The urea clearance tended to return to the control value within one week.

The diet was changed to Cero-Meato at this time. After allowing about one month for complete recovery from ether and for adjustment to the new diet, new controls were established and additional studies were made. Creatinine clearances were begun and the two clearance tests were done simultaneously. According to Jolliffe,[8] the administration of creatinine does not affect the rate of urea excretion.

(d) Sodium amytal (Diet—Cero-Meato)

Eighteen periods of anesthesia on Dogs C, D, K, and L were observed. The following results were found:

	UREA CLEARANCE		CREATININE CLEARANCE	
DOG	CONTROL	AFTER ANESTHESIA	CONTROL	AFTER ANESTHESIA
C	68.9	68.9	75.9	91.3
D	50.4	62.5	59.5	69.3
K	64.3	72.9	75.8	107.8
L	71.3	49.8	74.3	86.7

As noted, the urea clearance did not show a constant change. The creatinine clearance increased in every case. The autopsy reports on these dogs are instructive.

1. Dog C was brought to autopsy after having had 52 urea clearance and 20 creatinine clearance tests. The glomeruli of the left kidney showed a thickening of the basement membranes of the capillaries, Bowman's capsules, and afferent arterioles. Many of these arterioles were dilated and the walls thickened where they entered the glomerular tuft. The basement membrane of the tubules was slightly thickened. There were thin-walled spaces lined by endothelium and containing blood scattered throughout the kidney substance and pelvis.

The right kidney showed intense congestion and hemorrhage. There was a triangular area infiltrated with mononuclear cells beneath the capsule. This area was partly fibrosed. The afferent arterioles and basement membranes were less affected than in the left kidney, but inflammatory lesions were more prominent. The diagnosis was interstitial nephritis and nephrosclerosis.

This dog showed relatively high clearances in spite of the renal pathology, but the variations after anesthesia were less marked than in some of the other dogs.

2. Dog D was brought to autopsy after 54 urea clearance and 27 creatinine clearance tests. The only report on the kidneys recorded by the pathologist was that the glomeruli were intensely congested and there was slight cloudy swelling of the tubules.

3. Dog K was brought to autopsy after 19 urea clearance and 18 creatinine clearance tests. The blood vessels in the interstitial tissue and some glomeruli were congested. There was a slight thickening of the basement membrane of the capillaries of the glomeruli and also a thickening and dilatation of some of the afferent arterioles. Many Bowman's capsules were thickened, and in some areas the capsules were enormously distended with granular material. These glomeruli resembled those seen in lipoid nephrosis in human kidneys. Cloudy swelling of the convoluted tubules was noted.

This dog had a high white blood cell count, as shown in Table I, and gave very irregular clearance tests, as might be expected in view of the renal pathology.

4. Dog L was brought to autopsy after 7 urea clearance and 7 creatinine clearance tests. The kidneys were intensely congested. The glomerular capillaries were enormously distended, with an occasional hemorrhage into the capsule. The appearance of some of the glomeruli suggested a hemorrhagic nephritis. The clearances were irregular in this dog.

(e) Sodium barbital (Diet—Cero-Meato)

Dogs M and F were used, and only four periods of anesthesia were observed. The following results were found:

	UREA CLEARANCE		CREATININE CLEARANCE	
DOG	CONTROL	AFTER ANESTHESIA	CONTROL	AFTER ANESTHESIA
M	59.5	61.8	77.1	53.1
F	60.6	46.5	58.8	85.5

As noted, the results were very irregular. Dog M, after 10 clearance tests, was used for other purposes and did not come to autopsy.

Dog F had been used for 59 urea clearance and 24 creatinine clearance tests before coming to autopsy. The kidneys showed congestion. In many glomeruli there was extreme dilatation of the afferent arterioles and the capillaries. Hemorrhage was evident in some of Bowman's capsules.

Dog B was used only for 8 control urea clearance tests. Since the range was from 35.7 to 132.3, it was used for other purposes and was not brought to autopsy.

SUMMARY AND CONCLUSIONS

1. The urea clearance increased as much as 40 per cent on the day following the administration of cyclopropane and returned to normal within a week in a dog with no renal pathology. In a dog with hypertension, high blood urea nitrogen, and some renal pathology, a fixed urea clearance was found.

2. The administration of pentobarbital sodium (Abbott) failed to produce greater changes in the urea clearance than were found in the normal range of two of the dogs.

3. The administration of ether (drop method) after a sedative dose of pentobarbital sodium was followed by a marked increase in the urea clearance in two dogs and a very slight increase in a third dog.

4. The administration of sodium amytal was followed by an increase in the urea clearance in one dog with little renal pathology; the results were inconstant in three dogs with considerable renal pathology. The creatinine clearance increased in every case; there was great variation in the amount of increase.

5. The administration of sodium barbital was followed by a decrease in the urea clearance and an increase in the creatinine clearance in one dog with some renal pathology.

6. High leucocyte counts, elevation of rectal temperature, and renal pathology seem to be associated with inconstant urea clearance.

7. The inconstancy of urea clearance results has been noted by Goldring,[9] Holman,[10] and Van Slyke.[11] We consider it worth while to follow a series of clearance tests on one animal by autopsy.

8. It is possible that sedation with barbiturates on the night preceding a clearance test may influence the results. An elevation of temperature and a high leucocyte count may likewise affect the results.

REFERENCES

1. Orth, O. S., and Stutzman, J. W.: Constancy of Urea Clearances in Dogs Following Surgical Anesthesias With Cyclopropane, Ether and Chloroform, Proc. Soc. Exper. Biol. & Med. **39**: 403, 1938.
2. Rhoads, C. P., Alving, A., Hiller, A., and Van Slyke, D. D.: The Functional Effect of Explanting One Kidney and Removing the Other, Am. J. Physiol. **109**: 329, 1934.
3. Ralli, E. P., Brown, M., and Pariente, A.: The Urea Clearance Test in Normal Dogs, Am. J. Physiol. **97**: 432, 1931.
4. Allen, F. M.: Auscultatory Estimation of Blood Pressure in Dogs, J. M. Research **4**: 431, 1923.
5. Van Slyke, D. D., and Kugel, V. H.: Improvements in Manometric Micro-Kjeldahl and Blood Urea Methods, J. Biol. Chem. **102**: 489, 1933.
6. Holten, C., and Rehberg, P. B.: Studies on Pathological Function of Kidneys in Renal Disease, Especially Bright's Disease, Acta Med. Scandinav. **74**: 479, 1931.
7. Hafkesbring, R., Greisheimer, E., and Magalhaes, H.: Effects of Repeated Anesthetic Doses of Barbiturates, J. Pharmacol. & Exper. Therap. **66**: 95, 1939.

8. Jolliffe, N., and Smith, H. W.: The Excretion of Urine in the Dog. I. The Urea and Creatinine Clearances on a Mixed Diet, Am. J. Physiol. **98**: 572, 1931.
9. Goldring, W.: Studies of the Kidney in Acute Infection. II. Observations With the Urea Clearance Test in Acute Rheumatic Infection, J. Clin. Investigation **10**: 345, 1931.
10. Holman, R. L.: Observations on the Urea Clearance in Dogs, Am. J. Physiol. **104**: 615, 1933.
11. Van Slyke, D. D., Rhoads, C. P., Hiller, A., and Alving, A.: Relationships Between Urea Excretion, Renal Blood Flow, Renal Oxygen Consumption and Diuresis. The Mechanism of Urea Excretion, Am. J. Physiol. **109**: 336, 1934.

INCIDENCE OF BACTERIEMIA IN PATIENTS WITH DENTAL DISEASE*

MAYNARD MURRAY, M.D., AND FRANK MOOSNICK, B.S.
CINCINNATI, OHIO

FROM clinical observation one is aware of the fact that bacteriemia must occur frequently without peripheral manifestations of sufficient grade to say the patient is "diseased." Proof may be seen in the fact that traumatized tissues in which abrasion of the skin was absent are frequently infected. Thus hematomas that come up infected or the osteomyelitides that follow slight trauma may be taken as good evidence that a bacteriemia existed and that an infecting organism reached the point of infection through the blood stream. As promulgated by Rosenow[1] and Fischer,[2] the teeth act as chronic foci, are a common source of invasion of the blood stream, and hence are responsible for the diseased processes that occur in distant lying tissues.

If the view of these authors is correct, one should expect to be able to uncover the existence of bacteriemias in patients affected with chronic dental disease. Okell and Elliot[3] found a transitory bacteriemia in from 60 to 70 per cent of their patients after the extraction of teeth under general anesthesia; Burket and Burn,[4] in 17 per cent of their patients when local anesthesia only was employed. The latter explained their low incidence by saying that the suprarenin employed acted as a vasoconstrictor to render the vessels less permeable to the entrance of microorganisms.

To learn how frequently bacteriemia occurs in dentally affected patients not subjected to operation, the following experiment was performed: Three hundred and thirty-six patients with varying degrees of dental caries and pyorrhea were asked to chew a 1 inch cube of high melting point paraffin. After chewing this for one-half hour, blood cultures were taken from the femoral artery.[5] (The area was scrubbed with tincture of green soap and water, followed by iodine and alcohol, and then the application of tincture of merthiolate. Cultures from skin areas so treated were routinely negative.) The blood withdrawn from the artery was mixed with brain broth containing one-half of 1

*From the Laboratory of Physiology, University of Cincinnati and Longview State Hospital, Cincinnati.
Received for publication, December 28, 1939.

per cent peptone, and allowed to stand for six days at 37° C. Thereafter subcultures were prepared in heart infusion agar.

These blood cultures proved positive immediately after chewing in 185, or 55 per cent, of the patients studied. Follow-up blood cultures were not taken to determine how long such a bacteriemia might persist.

The organisms found were a small diplococcus in 84 per cent of the positive cultures and a slowly growing *Staphylococcus albus* in the remaining 16 per cent. One patient displayed both organisms. Subclassification was not completed.

SUMMARY

Of 336 patients with dental disease, 185 gave positive arterial blood cultures after they had chewed paraffin for one-half hour.

REFERENCES

1. Rosenow, E. C.: Elective Localization of Streptococci, J. A. M. A. 65: 1687, 1915.
2. Fischer, Martin H.: Personal communication.
3. Okell, C. C., and Elliot, S. D.: Bacteremia and Oral Sepsis With Special Reference to the Etiology of Subacute Endocarditis. Bacteremia Following Dental Extractions, Lancet 2: 869, 1935.
4. Burket, Lester W., and Burn, Casper G.: Bacteremias Following Dental Extraction, J. Dent. Research 16: 521, 1937.
5. Murray, Maynard, and Moosnick, Frank: Simultaneous Arterial and Venous Blood Cultures, J. Lab. & Clin. Med. 26: 382, 1940.

THE HISTAMINE TREATMENT OF ALLERGIC DISEASES*

I. Asthma and Vasomotor Rhinitis

Laurence Farmer, M.D., New York, N. Y.

NUMEROUS observations have led to the assumption that the symptoms of experimental anaphylaxis and of clinical allergy are caused by histamine or a histamine-like substance. Dale's[1] theory of anaphylaxis presupposes that the combining of antigen and antibody in sensitized tissues leads to cell lesion and the liberation of preformed histamine or of a histamine-like substance. Lewis and his collaborators Grant and Hare[2, 3] expanded this theory to explain the "anaphylactic" skin reactions in allergic human beings. In certain individuals heat, cold, or mechanical irritation, in degrees perfectly tolerated by normal persons, causes cell lesion and the liberation of histamine from the tissues (Duke, Bray, Horton[4]). The clinical manifestations of these "physical" allergies are usually the same as those in the "atopic" allergies.

Fuehner[5] was the first to show that it is possible to induce refractoriness to histamine in normal animals. This phenomenon was observed in normal human beings by Lewis and Grant.[6] Hare showed in allergic persons that "the histamine stimulus renders the skin refractory to the anaphylactic poison."

*From the Allergy Clinic, Lenox Hill Hospital, New York.
Received for publication, January 2, 1940.

Nonspecific "desensitization" with histamine was achieved by Farmer,[7] who was able to induce diminished sensitiveness to serum in serum-sensitized guinea pigs through oral and parenteral application of histamine.

The theoretical and practical implications of these observations for the understanding and treatment of allergic diseases are obvious. The histamine theory would explain why the symptoms of allergic conditions are independent of the nature of the causative allergen, and dependent only on the reaction of the shock organs. The possibility of achieving refractoriness to histamine would reduce the treatment of allergic diseases to a common denominator and thereby make for much simplification. It would, furthermore, be very useful in all those cases of multiple sensitiveness in which several allergens would have to be used for specific desensitization, and also in those cases in which the causative allergen or allergens could not be discovered.

Histamine was first used in the treatment of an allergic condition by Ramirez and St. George[8] in 1924. These authors had observed a group of patients with what they termed "endogenous" asthma. The patients who did not "as a rule react to the various proteins tested" but gave "an exceptionally strong reaction with solutions of histamine," were suffering, according to Ramirez and St. George, from "histamine sensitivity." Ten patients were treated with subcutaneous injections of histamine in doses ranging from 30 to 750 gamma; very good results were achieved in two patients.

The cases of Ramirez and St. George fit neither into the category of "atopic" nor into "physical" allergy. Recently Horton[9] has described a syndrome, which he calls "erythromelalgia of the head" (vascular headache), and which he attributes to histamine. Relief of the symptoms in these cases was also achieved by histamine injections. It is possible that the cases of Horton belong to the same category as those of Ramirez and St. George.

In 1927 Friedlaender and Petow[10] reported that they had used histamine in the treatment of migraine of various origins. Ernestene and Banks,[11] Gajdos,[12] and Joltrain[13] treated urticaria with histamine; and Collens and his collaborators[14] used it successfully in a diabetic person who had developed insulin sensitivity.

The reports on the use of histamine in bronchial asthma are few and far afield. In 1932 Stahl and Masson[15] in Strasbourg, and two years later Piquet,[16] Stahl's pupil, treated asthmatic attacks by applying histamine to skin scarifications. In 1935 Thiberge[17] in this country, and Dszinich[18] in Hungary reported on the treatment of asthma with injections of histamine. Thiberge used large doses of histamine: 0.5 c.c. of a 1:1,000 solution per injection. Dszinich used much smaller amounts, his initial doses in severe cases being 0.01 gamma, and in milder cases 0.1 gamma (0.00001 mg. and 0.0001 mg., respectively). Jacquelin[19] in 1937 stated that he had applied histamine by iontophoresis in a few cases of asthma. Openchowski[20] mentioned having used histamine in asthma and other allergic conditions, but he does not give any details. All these authors were impressed by the results they achieved.

In view of these favorable clinical reports and of the afore-mentioned theoretical considerations, we felt that the use of histamine in allergic diseases

TABLE I

CASE	AGE	DIAGNOSIS	YEARS OF ILLNESS	SEVERITY	RESULT OF SKIN TESTS	TREATMENT				RESULT
						1ST INJECTION γ	HIGHEST DOSE γ	TOTAL INJECTIONS	DURATION OF TREATMENT	
1. J. F. ♂	48	Asthma Rhinitis	36	Moderately severe	Milk ++ Feathers + Sheep wool +	0.1	30.0	31	12/16/38- 5/12/39	Marked improvement
2. J. C. ♂	30	Asthma	4	Moderately severe	Negative	0.1	15.0	24	12/ 7/38- 5/12/39	Marked improvement
3. F. L. ♂ (Baker)	36	Rhinitis	3	Severe	Wheat +++	0.1	40.0	24	2/21/38- 7/ 6/38	Very marked improvement
4. J. H. ♂	8	Asthma Rhinitis Eczema	4½ 7½	Moderately Severe	Rabbit dander + ?	0.01 0.1	2.5 0.8	17 8	3/30/38- 7/ 1/38 10/ 3/38-12/ 9/38	Very marked improvement
5. J. M. ♂	6	Asthma	2	Severe	House dust ++ Dog dander ++	0.01 0.01	0.1 0.5	7 21	11/ 5/37-11/24/37 3/ 9/38- 6/22/38	No improvement
6. M. M. ♀	28	Asthma	5	Severe	Negative	0.1 0.1	0.75 6.0	5 14	11/29/37-12/17/38 10/ 3/38- 8/12/38	No improvement
7. I. M. ♀	33	Asthma Rhinitis	5	Moderately severe	House dust ++ Dog dander ++	0.05	25.0	34	4/22/38- 9/ 9/38	Marked improvement
8. A. O. ♂	11	Asthma	6	Moderately severe	House dust ++ Sheep wool ++ Horse dander + Orris root +	0.05	10.0	15	11/29/37- 1/ 5/38	Very marked improvement
9. W. K. ♂	10	Asthma	4	Severe	House dust ++ Sheep wool ++ Feathers + Carrot + Lettuce +	0.02 0.02	1.0 0.1	16 19	1/30/39- 3/27/39 4/24/39- 6/21/39	No improvement
10. A. F. ♀	43	Asthma Rhinitis	2	Moderately severe	Negative	0.1	25.0	18	9/ 7/38-11/ 7/38	No improvement
11. B. S. ♀	41	Asthma Rhinitis	1	Severe	House dust + Feathers + Monilia ++ Ragweed ±	0.1 0.01	0.3 0.15	13 28	12/28/38- 2/ 3/39 2/24/39- 7/31/39	No improvement (condition worse) Improvement
12. H. J. ♂	33	Asthma Eczema	30	Moderately severe	House dust ++ Feathers + Sheep wool + Rabbit dander +	0.1 0.1 0.1	15.0 1.5 20.0	44 9 20	11/ 5/37- 7/ 1/38 12/ 5/38- 1/18/39 5/24/39- 8/16/39	Very marked improvement

TABLE I—CONT'D

13. R. B. ♂	2	Asthma	1	Moderately severe	Cottonseed +++	0.01	0.6	30	10/11/37- 7/13/38	Marked improvement
14. M. A. ♀	29	Rhinitis Eczema Hay fever	6 4 2	Moderately severe	House dust + Orris root + Ragweed + Prune ++ Bean + Potato + Tomato + Mustard +	0.1	3.5	13	1/31/38- 3/14/38	No improvement
15. M. R. ♀	21	Rhinitis	2	Moderately severe	Cornmeal + Tea leaf + ? Orange + ?	0.1	10.0	26	5/ 5/37- 7/26/37	Marked improvement
16. E. S. ♂ (Baker)	36	Asthma Rhinitis	2	Moderately severe	House dust + ? Feathers + ? Sheep wool + ? Wheat +++	0.1	5.0	17	1/26/38- 4/27/38	Marked improvement
17. K. F. ♀	54	Asthma	1½	Severe	Orris root ++ Cat dander + (+) Dog dander +	0.01 0.02	4.5 10.0	44 29	1/19/38- 7/ 6/38 9/27/38- 6/21/39	Improvement
18. H. V. ♂	7	Asthma Rhinitis	11½ 3	Moderately severe	House dust ++ Feathers +++ Rabbit dander +	0.01	4.0	28	1/20/39- 7/24/39	Very marked improvement
19. J. B. ♀	6	Asthma Hay fever	1	Moderately severe	Dog dander + (+) Rabbit dander ++ Feathers ++ Kapok + (+) Ragweed ++	0.01 0.01	1.6 0.7	15 12	11/ 4/38- 1/ 9/39 5/15/39- 7/10/39	Very marked iment
20. M. G. ♀	27	Asthma	2	Severe	Negative	0.01	0.6	14	2/ 8/39- 4/17/39	Very marked improvement
21. E. J. ♂	68	Asthma	50	Severe	Tobacco + Feathers ++	0.1	20.0	19	12/28/38- 4/28/39	Improvement
22. J. D. ♂	57	Asthma	8 mo.	Severe	Negative	0.05 0.1	20.0 20.0	37 28	12/16/37- 8/ 3/38 10/11/38- 6/13/39	Marked improvement
23. H. W. ♀	50	Asthma	30	Severe	Negative	0.01	3.0	20	1/ 9/39- 6/ 2/39	Very marked improvement

could be of essential value. It was necessary to investigate further the question of dosage and of appropriate mode of application. We felt that injection would be preferable to scarification or iontophoresis, as neither of these latter methods allows accurate dosage. However, we believed that the smaller amounts of histamine which were used in these methods, and which were also recommended by Dzsinich, would give better therapeutic results than the larger doses used by the earlier workers.

We have been using subcutaneous injections of histamine phosphate* in the treatment of various allergic diseases for three years. No hard and fast schedule of dosage has been developed. Following the suggestion of Dzsinich, the initial dose in mild cases of asthma is 0.1 gamma, and in more severe cases it is 0.01 gamma. The injections are increased each time by 50 per cent, if well tolerated, and in the beginning they are given two to three times weekly. They are later spaced at approximately five-, seven-, ten-, fourteen-, and twenty-one-day intervals. The rapidity with which the dose is increased and the spacing of the injections will depend upon the patient's tolerance and the results achieved. The maximum dose has been 50 to 75 gamma. However, in most instances very satisfactory therapeutic results have been achieved with much smaller amounts of histamine. If an injection is not well tolerated, or if a marked local reaction occurs, a smaller amount should be given the next time. In some instances, after marked improvement had been achieved in the course of treatment, the patient's condition gradually became worse. Since we felt that the patients were possibly getting too much histamine, we decreased the amount injected, and the patients again improved. In a number of cases even such small amounts of histamine as 0.03 to 0.07 gamma have caused marked exacerbation of the asthmatic attacks. It was necessary to reduce the injections to 0.005 gamma and to increase the doses very slowly. There is no method of ascertaining the amount of histamine needed in each individual case other than following the clinical course.

As in specific desensitization, histamine injections should be continued over a considerable period of time, especially if the therapeutic results are good. We have found it advisable to give a second or third series of injections after a pause of several months, even in the absence of symptoms.

We have given several thousand individual injections of histamine and have encountered a systemic reaction in only two instances. In the one case, after the injection of 1.0 gamma, the patient who was suffering from vasomotor rhinitis developed severe urticaria, which started at the site of the injection and spread over the entire body. The urticaria persisted for several days. In the other case the patient developed angioneurotic edema of the eyelids on several occasions after the injection of small amounts of histamine.

The precautions to be taken are the same as in specific desensitization. The injections should not be given into a venule or capillary, adrenalin and a tourniquet should always be on hand, and the patient should be observed for ten to fifteen minutes after the injection. However, we believe that histamine treatment causes systemic reactions less frequently than specific desensitization.

*Parke-Davis & Co. product.

We have treated with histamine 54 patients suffering from asthma, vasomotor rhinitis, or both, whose symptoms were in most instances due to allergens other than pollens. (We intend reporting on the pollen-sensitive patients in another paper.) Table I contains data on only 23 of the 54 patients. These patients were observed sufficiently long to allow conclusive evaluation of the effectiveness of histamine treatment.

All patients received physical and x-ray examinations, a routine urine examination, and a complete blood count; skin tests were carried out with the most important foodstuffs and inhalants. In almost all cases the patient was also examined otolaryngologically. As shown in Table I, the majority of the patients gave positive skin reactions to one or more allergens. These allergens were eliminated if possible, and the patient received histamine if the elimination did not lead to the desired therapeutic result.

CASE REPORTS

The following three case histories, which we present in detail, are illustrative of successfully treated patients:

CASE 3.—F. L. is a 36-year-old baker who has been suffering from severe vasomotor rhinitis for three years. His attacks of paroxysmal sneezing and profuse watery nasal discharge come on only in the bakeshop and only when working with wheat flour. They commence after he has been in the bakeshop for one to two hours, and last for many hours. His personal and familial histories are not contributory. Two years before he visited us he had a tonsillectomy; one and one-half year before, a resection of nasal septum with some improvement. X-ray of the chest and blood count were normal. Intradermal skin test with wheat extract was strongly positive. Histamine treatment was started with 0.1 gamma on Feb. 21, 1938. Decided improvement was noted after the eleventh injection, 3.0 gamma, on March 21. Improvement continued and the patient was able to work during April, May, and June without any symptoms of rhinitis. He was receiving injections of histamine during this time. The last injection of 40 gamma was given on July 6. The patient came back to the clinic on Sept. 21, 1938. He had been working during the summer and had had only infrequent, very mild symptoms. During the week prior to his readmission he had been sneezing more frequently. He was given four injections of histamine, and then did not return to the clinic as he was again feeling better. On July 28, 1939, he reported that he had been steadily working with wheat flour since Nov. 1, 1938, and that he had had no symptoms of rhinitis.

CASE 18.—H. V., a 7-year-old boy, has been suffering from rhinitis for three years and from bronchial asthma for one and a half years. His first attack of asthma occurred two weeks after tonsillectomy and adenoidectomy. The attacks come during the day and night, without any seasonal incidence. His mother has had eczema for eight years. Physical examination was negative; x-ray examination of the chest revealed slightly emphysematous lungs; differential blood count: 7 per cent eosinophiles; skin tests: house dust ++, feathers +++, rabbit dander +. Elimination of feathers from the child's environment brought no relief of symptoms. On Jan. 20, 1939, he was given his first injection of histamine, a 0.01 gamma dosage. The allergic symptoms became milder soon after the beginning of treatment. Coughing and sneezing persisted longer than the asthmatic attacks. In April the patient had an attack of asthma which lasted for three days. At that time he was given 15 injections of histamine (highest dose was 1.0 gamma). There was very marked improvement by the end of May. The last injection (twenty-eighth), 4.0 gamma, was given on July 17, 1939. The child had no allergic symptoms at that time.

CASE 23.—H. W., a 50-year-old woman, has had severe asthma for thirty years. Several attempts at specific desensitization have aggravated her condition considerably. The last

treatment (injections of house dust and feather extracts) had been given eight years prior to her admission to the clinic. The patient's condition had been steadily becoming worse and she was giving herself injections of adrenalin two to three times daily. The physical examination was negative with the exception of wheezing breath sounds. The blood count was normal. Skin tests were negative. Urethane, 2 to 4 Gm. daily, was given symptomatically,[21] with marked beneficial effect. The first injection of histamine (0.01 gamma) was given on Jan. 9, 1939. Under histamine treatment the patient's condition improved gradually. The attacks became less frequent and less severe. The patient was able to decrease the amount of adrenalin needed to stop the attacks. She had asthmatic attacks through January, but no attacks in February. By the beginning of March she had received 14 injections of histamine, the maximum dose at that time was 0.8 gamma. She had a moderately severe attack on March 3. There was occasional shortness of breath during March, but no real attacks of asthma. The patient from then on improved rapidly. Since she was planning to leave the city in June, the injections were given at twelve-day intervals during April, and at weekly intervals during May. She had no attacks during this time. The last injection (twenty-fifth), 3.0 gamma, was given on June 2. On August 17 she reported slight shortness of breath on two occasions since she was last seen. Her condition was very good and she needed no medication.

COMMENT

Our results with histamine treatment have been very encouraging, especially in a number of cases of asthma of long standing. One of the main problems still confronting us is the question of dosage. We feel that the earlier workers in this field were using too large amounts of histamine and that the dosage suggested by Dzsinich, which we used, is more appropriate.

We believe that some of our therapeutic observations, as for instance in the case of the wheat-sensitive baker, are a further proof of the correctness of the histamine theory of allergic conditions.

SUMMARY

1. The work of Dale and Lewis has laid the foundation for the theory that the symptoms of experimental anaphylaxis and of clinical allergy are due to the liberation of histamine or of a histamine-like substance in sensitive tissues.

2. Various authors have shown that it is possible to induce refractoriness to histamine in human beings and in laboratory animals. Furthermore, it has been demonstrated that it is possible to "desensitize" sensitized guinea pigs nonspecifically by injections of histamine.

3. These observations form the theoretical basis for the treatment of allergic diseases with histamine.

4. The technique of histamine treatment of asthma and vasomotor rhinitis is described, and the results of this treatment are discussed. The results have been very encouraging in a considerable number of instances and warrant further investigation.

REFERENCES

1. Dale, H. H.: Some Chemical Factors in the Control of the Circulation, Lancet **1**: 1285, 1929.
2. Lewis, T., and Grant, R. T.: Notes on the Anaphylactic Skin Reaction, Heart **13**: 219, 1926.
3. Hare, R.: An Experimental Investigation Into the Vascular Reactions of the Susceptible Skin to Protein, Heart **13**: 227, 1926.

4. Duke, W. W.: Urticaria Caused Specifically by Action of Physical Agents, J. A. M. A. **83**: 349, 1924.
Bray, G. W.: A Case of Physical Allergy, J. Allergy **3**: 367, 1932.
Horton, B. T., Brown, G. E., and Roth, G. M.: Hypersensitiveness to Cold, Etc., J. A. M. A. **107**: 1263, 1936.
5. Fuehner, H.: Das Pituitrin und seine wirksamen Bestandteile, München. med. Wchnschr. **59**: 852, 1912.
6. Lewis, T., and Grant, R. T.: Vascular Reactions of the Skin to Injury. II. Heart **11**: 209, 1924.
7. Farmer, L.: Non-specific "Desensitization" Through Histamine, J. Immunol. **36**: 37, 1939; **37**: 321, 1939.
8. Ramirez, M., and St. George, A. V.: A Contribution to the Etiology of Asthma, M. J. & Record, Jan. 16, 1924.
9. Horton, B. T., Mac Lean, A. R., and Craig, W. McK.: A New Syndrome of Vascular Headache, Proc. Staff Meet., Mayo Clin. **14**: 257, 1939.
10. Friedlaender, W., and Petow, H.: Migräne als Symptom von Cholange-Hepatopathien und ihre Behandlung mit Histamin, Med. Klin. **23**: 1498, 1927.
11. Ernestene, A. C., and Banks, B. M.: Use of Histamine in the Treatment of Pruritus, J. A. M. A., p. 328, 1933.
12. Gajdos, A.: "La réactogénothérapie de l'urticaire récidivant, J. d. practiciens **48**: 517, 1934.
13. Joltrain, E.: Histamine et urticaire, Progrès méd. p. 164, 1937.
14. Collens, W. S., Lerner, G., and Fialka, S. M.: Insulin Allergy. Treatment With Histamine, Am. J. M. Sc. **188**: 528, 1934.
15. Stahl, J., and Masson, J.: Histamine et dyspnées asthmatiformes, Compt. rend. Soc. de biol. **111**: 1003, 1932.
16. Piquet, E.: Asthme et Histamine, Thèse de doctorat, Strasbourg, 1934.
17. Thiberge, N. F.:. Histamine and Typhoid Protein Compared in the Control of Asthma and Hay Fever, J. Allergy **6**: 282, 1935.
18. Dzsinich, A.: Die Histaminbehandlung allergischer Zustände, Klin. Wchnschr. **14**: 1612, 1935.
19. Jacquelin, A.: Asthme et histamine, Progrès méd. p. 170, 1937.
20. Openchowski, M.: Histamine—Its Practical Application and the Theory of Its Action, J. M. Soc. New Jersey **34**: 163, 1937.
21. Farmer, L.: The Use of Urethane in Symptomatic Treatment of Bronchial Asthma, J. Lab. & Clin. Med. **24**: 453, 1939.

INVESTIGATIONS ON THE RETICULO-ENDOTHELIAL FUNCTION OF CANCER PATIENTS

Kurt Stern, M.D.,* New York, N. Y.

THE results of numerous experimental studies,[1] dealing with the role of the reticulo-endothelial system in cancer pathology, suggested to several research workers the possibility of their clinical application. Such investigations might prove of value in two ways: yielding an answer to the questions (a) whether or not a definite and regular involvement of the reticulo-endothelial system is associated with human neoplasia, and (b) if of a positive nature, this evaluation of the reticulo-endothelial function of cancer patients can be utilized for diagnostic and/or prognostic purposes.

Papers on this problem have been published by Krätschell,[2] Barbera,[3] Kavetskiy,[4] and Schroeder.[5] All these authors stated on the basis of their work that a definite damage of the reticulo-endothelial function could be found in

*This work was carried out as Research Fellow of the New York City Cancer Hospital and subsequently as Postgraduate Student Observer of the New York University Medical College.

Received for publication, February 3, 1940.

a high percentage of the tested cancer patients. The method generally used in these studies was the Congo red test proposed by Adler and Reimann.[6] This test is based on the fact that Congo red, a colloidal dye, is stored after intravenous injection by the reticulo-endothelial cells. If, therefore, blood samples are withdrawn shortly after the intravenous injection and later after an interval of time, the concentration of the dye in the blood is found decreased in the second sample, thus permitting an estimate of the per cent of the dye which has been intercepted by the reticulo-endothelial system. The ratio between the dye concentrations in the two samples depends on the storing activity of the reticulo-endothelial system and is termed the Congo red index.

It must be admitted that objections have been raised against the usefulness of the Congo red test for an evaluation of the reticulo-endothelial function.[7, 8] But, on the other hand, animal experiments[9-11] definitely showed the direct connection of the functional activity of the reticulo-endothelial system and the Congo red test. Certainly the colloid-storing ability of this system is only one of its many functions, and it would be of great interest to include these in the clinical examination. But as these immunobiologic, metabolic, hormonal functions of complex character have not as yet been developed as generally applicable functional tests, the present available methods must be utilized. Besides Congo red, other colloids have been proposed for testing the storing capability of the reticulo-endothelial system,[12] but none of these methods appear to be superior to the Congo red test in simplicity and efficiency.

In previous investigations, in collaboration with Willheim,[13] we applied the Congo red test to rabbits and worked out a modified method which (a) required only small amounts of blood (0.4 c.c. for the single sample, in contrast to the 10 to 20 c.c. of blood necessary in the original method), and (b) permitted a more exact colorimetry by transforming the Congo red into the acid, blue form. Later we published a similar modification[14] which is applicable for clinical use and serves to the same advantage. The usefulness of these modifications has been confirmed by other authors who have used them in animal experiments[15] and for clinical purposes.[16]

Because the original studies of the association of reticulo-endothelial function with cancer were carried out on a comparatively small number of cases—in about 50 patients for each experiment—and because we now have at our disposal a more reliable modification of the Congo red test which is also less disturbing to the patient, we were induced to resume the study of this problem and to apply this functional test of the reticulo-endothelial system to a larger group of cancer patients.

Method.—The original test, as well as our modification, is based on the following principle: 10 c.c. of 1 per cent watery Congo red solution are injected intravenously, four and sixty minutes, respectively, after the injection blood samples are withdrawn, and in the serum or plasma the Congo red concentration is colorimetrically determined. As the standard required for the colorimetry must contain the same amount of the patient's serum or plasma, another blood sample is first obtained immediately before the injection of the dye. While in the original method a rather primitive comparative estimation of the red color

of the samples was made, in our modification the Congo red is transformed into the blue compound by addition of hydrochloric acid, before which an acid buffer solution, containing urea, was added, thus (1) diminishing the acid-binding capacity of the serum proteins and (2) preventing their flocculation. Because of the low dye concentration in the samples (2 to 5 milligrams per cent, i.e., 20 to 50 γ in 1 c.c.), it was found necessary for exact colorimetry to add measured amounts of Congo red to the samples. By these procedures clear blue violet hues are obtained which can be easily colorimetrically compared and are proportional to the dye concentration as control experiments proved.

Solutions.—(1) A 1 per cent watery solution can be prepared by exactly weighing Congo red powder and sterilizing in the autoclave. This solution should be used within twenty-four hours and, therefore, must be freshly prepared. For the majority of our experiments we used Loeser's commercial preparation of "1 per cent Congo red."

(2) Sterile 3.8 per cent solution of sodium citrate.

(3) One-tenth per cent and 0.05 per cent Congo red solutions, freshly prepared by dilution of the 1 per cent Congo red solution, used for the injection in the same experiment.

(4) Glycine-hydrochloric acid buffer, containing 25 per cent urea. A mixture of 0.75 Gm. of glycine and 0.59 Gm. of sodium chloride are dissolved in 100 c.c. of water, and 52 parts of this solution are mixed with 48 parts of tenth-normal hydrochloric acid. In 100 c.c. of this mixture 25 Gm. of urea are dissolved. This reagent has a pH of approximately 2, and is usable for several weeks, if stored in the icebox.

(5) Ten per cent hydrochloric acid.

Performance of the Test.—To avoid lipemia of the blood samples, the tested patient should be bled either after fasting or at least not shortly after the intake of food.

1. Using a dry 5 c.c. syringe or one rinsed with citrate solution, containing exactly 1 c.c. of 3.8 per cent sodium citrate, blood is aspirated from the cubital vein until the 5 c.c. mark (4 c.c. of blood). Aspiration of air bubbles must be avoided. The contents of the syringe are mixed and put into a dry centrifuging tube, marked *Sample 1.*

2. Immediately following, while the needle remains in the vein, 10 c.c. of the 1 per cent Congo red solution are injected.

3. Four minutes after the injection blood is withdrawn in the same way as described above (*Sample 2*).

4. *Sample 3* is obtained sixty minutes after the injection.

Colorimetry.—All three samples are sharply centrifuged. After careful separation from the red blood cells, 2 c.c. of each sample are put into dry test tubes. To the colorless sample 1, serving as standard, 0.1 c.c. of the 0.1 per cent Congo red solution is added with a micropipette; to samples 2 and 3, 0.1 c.c. of the 0.05 per cent Congo red solution, corresponding to 100 and 50 γ, respectively, is added. To each sample is now added 2 c.c. of the buffer-urea solution and 0.5 c.c. of 10 per cent hydrochloric acid. Thus a blue violet color is

developed and the colorimetry is immediately performed, as precipitations may occur after longer delay. Sample 1, containing 100 γ Congo red, is used as standard.

Calculation.—The Congo red content of samples 2 and 3 is found by the equation $x = \frac{1000}{r}\gamma$, if the standard is kept at the mark 10 and r is substituted by the respective reading. From these values, 50 γ, which have been added as previously described, are subtracted for obtaining the originally present Congo red content of the samples. The Congo red index, showing the per cent of decrease of the Congo red and thus serving as measure of the reticulo-endothelial activity, is obtained by dividing the Congo red content of sample 3 by the Congo red content of sample 2 and multiplying this figure by 100.* The Congo red content of the samples, expressed in milligrams per cent, is found by dividing the γ values by 16.

TABLE I

CANCER-FREE PATIENTS

NO.	AGE	DIAGNOSIS	C.R.I.*	NO.	AGE	DIAGNOSIS	C.R.I.*
1	38	Sinusitis	44.9	26	28	Eczema	66.8
2	63	Lipoma of neck	37.6	27	60	Mycosis	63.9
3	39	Ulcer of breast	34.3	28	32	Warts	57.5
4	39	Keloid of ear	33.2	29	37	Menorrhagia	69.9
5	37	Syringomyelia	50.4	30	48	Metrorrhagia	49.6
6	22	Fibroma of nose	56.8	31	21	Acne vulgaris	57.8
7	30	Healthy	45.1	32	71	Dermatitis	65.5
8	48	Eczema	54.6	33	33	Keloid of arm	67.1
9	26	Keloid of ear	57.5	34	57	Warts	55.6
10	60	Neuralgia	62.5	35	56	Endometritis	34.8
11	44	Sebaceous cyst	67.5	36	48	Eczema	62.8
12	73	Morbus frey	62.9	37	24	Keloid of breast	63.1
13	45	Cellulitis of leg	61.3	38	21	Ringworm	52.8
14	39	Sebaceous cyst	63.5	39	28	Eczema	64.1
15	28	Keloid of ear	33.3	40	28	Tuberculosis of rib	60.5
16	45	Eczema	61.3	41	56	Metrorrhagia	73.9
17	39	Boils	32.7	42	59	Fibroid of uterus	52.8
18	37	Psoriasis	62.1	43	58	Dermatitis	35.2
19	43	Herpes zoster	59.8	44	38	Fracture of tibia	56.5
20	64	Dupuytren's contracture	57.1	45	47	Fracture of femur	57.1
21	21	Epidermophytosis	34.8	46	27	Spinal cord lesion	61.9
22	45	Dermatitis	69.7	47	46	Fracture of tibia	51.6
23	67	Mycosis	37.7	48	27	Hallux valgus	69.9
24	44	Mycosis	44.4	49	61	Fracture of femur	50.2
25	20	Acne vulgaris	67.6				

*Congo red index.

Complications.—By strict attention to the above described procedure the plasmas obtained are never hemolytic. Should hemolysis occur, however, this source of error, so important in the original method, is easily detected as the acidification with hydrochloric acid results in a brownish color (hematin), which does not allow a colorimetric comparison. Such a test should be discarded. Strongly icteric or lipemic samples also might prevent a successful colorimetric determination. Finally, it is known that in amyloidosis intravenously injected

*Some authors, ourselves included, in reported animal experiments used a different Congo red index, figuring the per cent of decrease according to the formula: Congo red content of sample 2 minus Congo red content of sample 3 divided by Congo red content of sample 2 and multiplied by 100. This value corresponds to 100 minus the original Congo red index and is the higher, the more Congo red has disappeared.

Congo red disappears very quickly from the blood stream because of a great chemical affinity of Congo red to amyloid (Bennhold's test). In these cases, of course, the Congo red test cannot be used for an evaluation of the reticulo-endothelial function.

Using the described method, we tested 49 patients clinically free of malignancy, and 100 patients suffering from malignant neoplasms. In 12 cancer patients the test was repeatedly performed during and after radiation therapy. The results of these tests may be seen from Tables I to IV.

Table I, comprising the cancer-free patients, exhibits values of the Congo red index ranging from 32.7 to 73.9 per cent. Eight patients (11, 22, 25, 26, 29, 33, 41, 48) show indices near or beyond 70 and correspondingly must be considered as more or less impaired in their reticulo-endothelial function. The normal range varies between 30 and 65 per cent. In our experiments the average value is found as approximately 55 per cent, which means that a little less than half of the injected Congo red amount is stored by the reticulo-endothelial system during the time of the test.

Turning to the neoplastic cases it can be seen that 86 out of the 100 tested patients found in Table II exhibit values ranging between 70 and 100. In 14 cases, however, included in Table III, the Congo red index was found below 70.

On the basis of these results it seems possible to draw the following conclusions:

1. In 86 per cent of the investigated cancer cases a definite severe damage of the reticulo-endothelial function has been found by means of the Congo red test. This damage does not depend on a disturbance of the patient's general condition nor does it seem to be connected with the advancement or the generalization of the malignancy. While at present it cannot be explained why in 14 per cent of our cases this phenomenon could not be observed, the marked involvement of the reticulo-endothelial system in human neoplasia may be considered as an established and probably important factor of cancer pathology. For the time being, however, it cannot be decided if this phenomenon is primarily connected with tumor origin and growth or is only a secondary, concomitant factor. An answer to this question may be looked for from suitable animal experiments.[17]

2. A diagnostic value cannot be attributed to the Congo red test both because of its nonspecificity as many other conditions, mainly infections, also result in damaged reticulo-endothelial function, and because in about 14 per cent of our cancer patients the Congo red test failed to reveal an impairment of the reticulo-endothelial system. Therefore, the result of the Congo red test might be evaluated only in combination with other clinical and laboratory findings.

3. On the other hand, a certain prognostic value seems to adhere to the test. By grouping the patients according to their Congo red index and comparing the occurrence of death within the time of observation (six to nine months), we see the following distribution:

Group I:	C.R.I. 70- 80	6 out of 20 patients died (30 per cent)
Group II:	C.R.I. 80- 90	21 out of 39 patients died (54 per cent)
Group III:	C.R.I. 90-100	19 out of 27 patients died (70 per cent)

TABLE II

CANCER PATIENTS WITH DAMAGED RETICULO-ENDOTHELIAL FUNCTION

NO.	DATE*	AGE	CANCER OF	C.R.I.	NOTE
1	12/22	45	Stomach	84.7	Died 12/29
2	12/23	78	Rectum	82.4	Unimproved 10/3
3	12/27	38	Rectum	76.1	Died 5/28
4	12/28	68	Prostate	86.2	Unimproved 10/3
5	1/ 3	78	Prostate	87.0	Improved 10/15
6	1/ 5	53	Floor of mouth	91.4	Died 6/17; cf. Table IV
7	1/ 6	68	Nasopharynx	75.2	Discharged unimproved 1/28
8	1/ 9	38	Perineum	88.4	Unimproved 10/3; cf. Table IV
9	1/ 9	64	Tongue	88.0	Died 8/7; cf. Table IV
10	1/10	78	Stomach	100.0	Died 3/19
11	1/11	68	Prostate	88.5	Died 3/6
12	1/12	41	Esophagus	88.3	Died 1/17
13	1/13	38	Breast	95.9	Died 7/4; cf. Table IV
14	1/17	28	Breast	84.6	Died 6/12
15	1/18	53	Rectum	86.7	Improved 9/26
16	1/18	43	Cervix	90.4	Unimproved 10/23
17	1/23	51	Esophagus	90.3	Discharged unimproved 1/31
18	1/24	45	Cervix	92.3	Died 3/29
19	1/25	45	Breast	89.3	Died 3/7
20	1/26	70	Rectum	83.1	Unimproved 12/22
21	1/26	78	Tongue	96.0	Discharged improved 6/24
22	1/27	73	Bladder	81.9	Unimproved 10/4
23	2/ 6	45	Larynx	71.0	Discharged unimproved 2/23
24	2/ 6	68	Tongue	90.5	Died 4/24; cf. Table IV
25	2/ 8	55	Breast	90.7	Died 8/20
26	2/ 9	52	Stomach	94.1	Died 7/2
27	2/ 9	74	Floor of mouth	100.0	Died 10/28; cf. Table IV
28	2/14	60	Stomach	87.5	Died 7/28
29	2/16	72	Tongue	73.7	Improved 5/5
30	2/16	49	Rectum	84.9	Unimproved 2/23
31	2/16	72	Larynx	83.4	Died 11/30
32	2/16	58	Penis	80.1	Died 3/20
33	2/21	46	Larynx	85.9	Died 5/26
34	2/21	58	Larynx	83.4	Unimproved 12/24
35	2/23	70	Rectum	72.6	Discharged unimproved 2/26
36	2/23	62	Lung	83.4	Died 4/13
37	2/23	71	Neck	94.1	Improved 7/6; cf. Table IV
38	2/27	59	Rectum	72.5	Died 10/24
39	2/27	62	Esophagus	94.2	Died 7/23
40	2/28	35	Neck	78.3	Improved 12/26
41	2/28	43	Cervix	95.7	Discharged improved 3/29
42	3/ 1	55	Breast	85.2	Died 5/22
43	3/ 2	69	Prostate	100.0	Died 3/16
44	3/ 2	44	Testis	87.1	Died 3/30
45	3/ 3	46	Larynx	75.7	Discharged improved 5/20
46	3/ 6	76	Breast	84.4	Discharged unimproved 3/11
47	3/ 7	59	Lung	81.8	Died 3/29
48	3/ 9	44	Tonsil	85.2	Died 3/23
49	3/ 9	58	Esophagus	85.3	Died 5/11
50	3/ 9	71	Prostate	90.5	Died 4/9
51	3/10	44	Cervix	80.9	Discharged improved 6/19
52	3/13	42	Breast	72.5	Discharged improved 4/21
53	3/13	59	Cervix	100.0	Died 4/19
54	3/14	69	Stomach	86.7	Discharged unimproved 4/1
55	3/17	70	Stomach	82.6	Discharged unimproved 4/6
56	3/20	66	Larynx	82.2	Discharged improved 4/24
57	3/21	64	Rectum	77.5	Died 4/14
58	3/21	78	Tongue	100.0	Died 4/9
59	3/23	77	Tonsil	85.8	Died 6/24
60	3/27	44	Face	75.4	Unimproved 10/29
61	3/27	84	Larynx	100.0	Died 8/24

TABLE II—CONT'D

NO.	DATE*	AGE	CANCER OF	C.R.I.	NOTE
62	3/28	36	Cervix	78.6	Died 6/24
63	3/30	52	Esophagus	100.0	Died 11/23; cf. Table IV
64	3/31	52	Rectum	92.3	Unimproved 12/23
65	4/ 6	47	Middle ear	90.3	Died 10/29
66	4/ 7	50	Tongue	88.5	Died 7/27; cf. Table IV
67	4/12	51	Tongue	96.9	Discharged improved 7/8
68	4/13	54	Kidney	79.9	Died 8/17
69	4/13	68	Cervix	72.8	Discharged unimproved 7/6
70	4/13	50	Cervix	90.1	Died 10/30
71	4/14	60	Rectum	88.7	Died 4/30
72	4/17	63	Prostate	96.0	Died 5/5
73	4/19	44	Lip	72.7	Improved 7/13
74	4/25	69	Stomach	84.0	Unimproved 10/24
75	4/26	57	Breast	82.3	Unimproved 10/24
76	4/26	62	Lip	71.5	Discharged improved July; cf. Table IV
77	5/ 3	28	Breast	77.1	No follow-up
78	5/ 4	50	Breast	85.4	Unimproved 7/20
79	5/ 8	89	Face	70.5	Unimproved 10/29
80	5/15	46	Breast	86.8	Discharged unimproved 10/1
81	5/25	58	Cheek	81.7	Died 8/19
82	6/21	56	Foot	90.1	Unimproved 12/18
83	6/22	54	Larynx	79.6	Unimproved 12/15
84	6/30	59	Lung	100.0	Died 7/18
85	6/30	61	Prostate	78.0	Unimproved 12/7
86	6/30	35	Lung	85.2	Died 7/13

TABLE III

"NEGATIVE" CANCER PATIENTS

NO.	DATE*	AGE	CANCER OF	C.R.I.	NOTE
1	1/12	56	Sigmoid	68.9	Improved 12/26
2	1/16	38	Cervix	66.8	Died 2/15
3	1/17	23	Cervix	56.5	Discharged improved 2/15
4	1/31	57	Larynx	58.6	Died 6/2
5	2/ 6	38	Lung	46.2	Discharged improved 6/23
6	2/ 7	50	Ovary	64.4	Discharged unimproved 2/15
7	2/15	48	Cervix	61.9	Died 4/29
8	3/ 1	53	Breast	46.3	Discharged unimproved 6/6
9	3/ 3	65	Cheek	67.2	Died 4/23
10	3/15	67	Colon	68.8	Died 6/22
11	3/15	59	Rectum	52.3	Died 3/18
12	3/16	38	Breast	66.6	Died 6/13
13	4/24	51	Tongue	60.0	Improved 12/13
14	5/10	49	Lip	65.3	Improved 12/19

*All dates refer to 1939 with exception of patients 1 to 4.

Therefore, the more severe disturbance of the reticulo-endothelial system appears to be connected with a more rapid fatal progress of the disease.

4. As shown in Table IV, the Congo red test was also repeatedly carried out in 12 cancer patients before, during, and after their treatment with radiation therapy. Here we see that in 2 patients (21, 37) the clinical improvement, achieved by the therapy, was also accompanied by a reversion of the Congo red index to normal values. In 2 patients (27, 66) the Congo red index was lowered, coinciding with a clinically observed radiation reaction and indicating an improved function of the reticulo-endothelial system, but no lasting clinical improvement was brought about. In the rest of the patients no clinical improvement was observed and the repeated performance of the Congo red test always

exhibited a damage of the reticulo-endothelial function. In patient 13 the regression of a breast lesion was also expressed by an improved reticulo-endothelial function, but the finally lethal recurrence was foreshadowed by an increase of the Congo red index, characteristic for the failure of the reticulo-endothelial function. As far as these few investigations permit us to judge, the repeated performance of the Congo red test during a treatment would prove valuable for prognostic purposes. In this connection it may be mentioned that these results support the opinion that successful radation therapy is accompanied by an activation of the reticulo-endothelial system or is partly dependent on it (Caspari,[18] Lewin[19]).

TABLE IV

TESTS REPEATED DURING TREATMENT OF CANCER PATIENTS

NO.*	DATE	CANCER OF	C.R.I.	TREATMENT	NOTE
6	1/ 5 4/17	Floor of mouth	91.4 90.6	X-ray: 1/8-3/28	Died 6/17
8	1/ 9 3/30 6/12	Perineum	88.4 83.1 94.2	X-ray: 4/30-6/28	Biopsy 5/9, malignancy present; identical finding 8/8
9	1/ 9 3/23	Tongue	88.0 87.8	X-ray: 2/17-3/28	Died 8/7
13	1/13 3/ 6 6/13	Breast	95.9 72.3 88.6	X-ray: 12/16, 38-6/28	Local condition healed 2/28; recurrence 6/30; died 7/4
21	1/26 3/ 6 6/12	Tongue	96.0 88.8 65.6	X-ray: 1/26-3/20; 4/10-4/20	Discharged improved 6/24
24	2/ 6 3/ 8	Tongue	90.5 86.3	X-ray: 2/23-3/29	Died 4/24
27	2/ 9 3/22 6/ 8	Floor of mouth	100.0 54.3 62.6	X-ray 2/15-3/15; radium mold: 5/15-5/19	Height of radiation reaction 3/22; marked regression 6/26; died 10/28
37	2/23 3/27 6/ 8	Neck	94.1 80.6 40.9	X-ray: 2/27-3/21; 5/24-6/25	Discharged highly improved 7/6
63	3/30 6/ 6	Esophagus	100.0 88.5	X-ray: 4/10-8/23	Died 11/23
66	4/ 7 6/ 8	Tongue	88.5 61.6	X-ray: 4/10/6/15	Died 7/27
76	4/26 6/14	Lip	71.5 74.8	Radium: May '39	Discharged improved July '39
78	5/ 4 6/14	Breast	85.4 71.5	X-ray: 5/10-6/15	No follow-up

*Numbers refer to corresponding cases in Table II.

5. In order to include a complete enumeration of our studies, we refer to the less conclusive results obtained by applying the Congo red test to 5 patients with sarcoma. Only in 2 of these was a decreased reticulo-endothelial function found: reticulum cell sarcoma, C.R.I. 85.8; fibromyxosarcoma, C.R.I. 100. In a case of fibrosarcoma of the intestines complete disappearance of the dye in the blood was observed after sixty minutes in a Congo red test performed twice (C.R.I. 0). No amyloidosis was found in the autopsy of this patient. In a patient with round-cell sarcoma a C.R.I. of 38.8, and in one with a reticulum-cell sarcoma a C.R.I. of 48.9 were found. While the number of the sarcoma patients studied is far too small to permit us drawing any conclusion, it does not seem

altogether improbable that the reticuloendothelial function is less frequently involved in sarcomas of mesodermal origin than in epithelial neoplasia. A similar view was expressed by Cramer[20] concerning the interpretation of reticulo-endothelial studies made on rat sarcomas.

SUMMARY

A modification of the Congo red test has been described which can be used for clinical testing of the reticulo-endothelial function. This test has been applied to 49 persons free of malignancy and to 100 patients afflicted with malignant neoplasms. A damaged function of the reticulo-endothelial system was found in 86 per cent of the cancer cases. The possibility of a prognostic evaluation of this test was discussed as to duration of life and effectiveness of radiation therapy.

REFERENCES

1. Willheim, R., and Stern, K.: Wege und Ergebnisse chemischer Krebsforschung, Vienna, 1936, Aeskulap-Verlag. pp. 355-378.
2. Krätschell, U.: Ueber das Verhalten des retikulo-endothelialen Systems bei malignen Tumoren an Hand der Kongorot Probe, Fortschr. d. Med. **51**: 983, 1933.
3. Barbera, S.: Esplorazione funzionale del sistema reticulo-endoteliale col rosso congo, Gazz. internaz. med.-chir. **45**: 710, 1935.
4. Kavetskiy, R. E.: Functional State of Connective Tissue During Cancerization Process, Med. zhur. **7**: 91, 1937.
5. Schroeder, W.: Ueber den Wert der Kongorot Probe bei Krebskranken, Klin. Wchnschr. **18**: 248, 1939.
6. Adler, H., and Reimann, F.: Beitrag zur Funktionspruefung des reticulo-endothelialen Apparates, Ztschr. f. d. ges. exper. Med. 47: 617, 1925.
7. Letterer, E.: Die Funktionspruefung mit Kongorot, Ztschr. f. d. ges. exper. Med. **93**: 103, 1934.
8. Ajello, L.: Sul valore della prova col rosso congo, Diag. e tec. di lab. **6**: 732, 1935.
9. Moncorps, C., and Guenther, O.: Chemische Identifikation einer aus der Milz isolierten, die mesenchymale Abfangfunktion steigernden Substanz als 2-oxy-6-aminopyrimidin (cytosin), Klin. Wchnschr. **12**: 979, 1933.
10. Goebel, F., and Miller, J. M.: L'ablation de la rate et le systéme réticulo-endothéliale, Compt. rend. Soc. de biol. **119**: 775, 1935.
11. del Zoppo, R.: La prova del rosso congo, Folia med. **21**: 33, 1935; Policlinico (sez. prat.) **42**: 89, 1935.
12. Brumann, H.: Methoden zur Untersuchung des R. E. S., Abderhalden's Arbeitsmethoden, Abtl. 8, Tl. 1, H. 7, Lief. 383.
13. Stern, K., and Willheim, R.: Beeinflussung des reticulo-endothelialen Systems durch Carotin, Ztschr. f. d. exper. Med. **97**: 354, 1935.
14. Stern, K.: Zur Methodik der klinischen Funktionspruefung des reticulo-endothelialen Systems mittels Kongorot, Wien. klin. Wchnschr. **50**: 1579, 1937.
15. Wetzler-Ligeti, C., and Wiesner, B. P.: Restropic Effects of Anterior Lobe Extracts, Endocrinology **22**: 693, 1938.
    Csato, T., Wetzler-Ligeti, C., and Wiesner, B. P.: Effect of the Prolonged Administration of 1:2:5:6-Dibenzanthracene on the Reticulo-Endothelial System of Rabbits, Am. J. Cancer **36**: 257, 1939.
16. Haas, F.: Unpublished study.
17. Stern, K.: Aspects on the Relationship Between Reticulo-Endothelial System and Malignancy, New Internat. Clin. In press.
18. Caspari, W.: Weiteres zur biologischen Grundlage der Strahlenwirkung, Strahlentherapie **18**: 17, 1924.
19. Lewin, C.: Die Immunitaetsvorgaenge bei malignen Tumoren, Ztschr. f. Krebsforsch. **27**: 138, 1928.
20. Cramer, H.: Unspezifische Krebstherapie, München. med. Wchnschr. **84**: 943, 1937.

VITAMIN K ACTIVITY OF 2-METHYL-1, 4-NAPHTHOQUINONE AND 4-AMINO-2-METHYL-1-NAPHTHOL IN HYPOPROTHROMBINEMIA*

E. A. SHARP, M.D., E. C. VONDER HEIDE, M.D., AND W. H. GOOD, M.D.
DETROIT, MICH.

IN VIEW of the promising preventive and curative role of vitamin K in hypoprothrombinemia, a brief communication is submitted on the recent chemical developments and the clinical status of synthetic vitamin K and substitute compounds.

MODE OF STUDY

Twenty-seven patients having hypoprothrombinemia below 40 per cent are reported. Specimens of blood were obtained by venipuncture during the morning hours. The prothrombin determinations were made by the method of Quick[1] and repeated at twenty-four-hour intervals when feasible. The normal clotting time for the method throughout the study was thirteen seconds, representing 100 per cent. Since many clinical variables must be considered in their eventual evaluation, this study involved only a preliminary assessment of activity of the chemical substances described when used in states of hypoprothrombinemia below 40 per cent of normal.

Dam[2] and Stokstad[3] are identified with the early phases of research on a factor having antihemorrhagic properties. The former demonstrated its presence in vegetables and alfalfa. Almquist and Stokstad[4] showed that a small percentage of alfalfa in the deficient diet prevented hemorrhagic tendency in chicks. Various contributions on the chemical nature of vitamin K cited by Doisy and his associates in a recent review[5] preceded the investigations of McKee, Binkley, Thayer, MacCorquodale, and Doisy,[6] and Binkley, MacCorquodale, Thayer, and Doisy[7] which led to the isolation of vitamins K_1 and K_2.

Almquist and Klose[8] reported on the antihemorrhagic property of a synthetic naphthoquinone, "phthiocol," 2-methyl-3-hydroxy-1, 4-naphthoquinone, which was isolated from the tubercle bacillus by Anderson and Newman[9] in 1933. These former workers considered "phthiocol" to be the simplest member of a homologous series of antihemorrhagic substances. They found it possessed the same degree of potency whether given orally, intramuscularly, or intravenously[10] but it had only 1/100 to 1/20 the activity of vitamin K derived from alfalfa.

In May, 1939, Doisy and his co-workers[11, 12] announced the isolation of two forms of vitamin K: K_1 from alfalfa and K_2 from fermented fish meal. As a

*From the Anemia Laboratory, Out-Patient Department, Harper Hospital, and the Department of Surgery, Wayne University, College of Medicine, Detroit.
Received for publication, February 13, 1940.

result of chemical analysis it was suggested that K_1 and K_2 possessed quinonoid structures. Further study demonstrated vitamin K to be disubstituted 1, 4-naphthoquinone.

Doisy and his co-workers assigned to vitamin K derived from alfalfa the chemical formula of 2-methyl-3-phytyl-1, 4-naphthoquinone. This substance has been synthesized and when used in this pure form orally has been found to induce remission of hypoprothrombinemia in animals and man. It is oil soluble and stable in oily solution, assaying 1,000 curative chick units* per milligram. While not fully evaluated clinically, a dose range of 6,000 to 9,000 units daily is effective when the prothrombin deficiency is below 40 per cent of normal.

Further research on substances having quinonoid structures by Ansbacher and Fernholz[13] demonstrated that 2-methyl-1, 4-naphthoquinone, first discovered by Fries and Lohmann in 1921,[14] possessed vitamin K activity. Each milligram of this substance assays about 2,000 curative chick units of vitamin K as determined by the seventy-two-hour method. It exists as bright yellow, prismatic crystals, possesses a quinone-like odor, and is affected by light; for example, exposure of the material in a glass-stoppered bottle to diffuse light for about two weeks not only changes the color to tan or off-white, but also raises the melting point from the normal range, 105° to 107° C., up to 170° C.

TABLE I

INEFFECTIVENESS OF 2-METHYL-1, 4-NAPHTHOQUINONE ORALLY IN DRY FORM IN HYPOPROTHROMBINEMIA OF OBSTRUCTIVE JAUNDICE AND BILIARY CIRRHOSIS

PATIENT NO.	PROTHROMBIN %		DAYS TREATED	DAILY DOSAGE (C.C.U.)*
	BEFORE	AFTER		
101	18	50	6	1,200
2Yo	18	25	11	2,400
3Za	40	50	7	2,400
3Za	40	50	9	7,200
4St	30	35	5	2,400
4St	30	35	7	3,600
4St	30	20	3	7,200
5Ba	40	20	14	2,400
6Mc†	15	22	3	3,600
7Po†	25	30	14	2,400

*Curative chick units.
†Biliary cirrhosis.

The compound 2-methyl-1, 4-naphthoquinone is soluble in oil and manifests antihemorrhagic action when given orally. Sharp[15] reported that doses of 1,200 to 7,200 curative chick units daily, when given orally in dry form, with or without whole bile, caused little alteration in hypoprothrombinemia associated with obstructive jaundice and biliary cirrhosis (Table I). When the same patients, however, received 2-methyl-1, 4-naphthoquinone dissolved in corn oil, a daily dosage of 2,400 to 4,800 units restored prothrombin to a satisfactory level within three to five days (Table II). Rhoads and Fliegelman[16] have reported favorably on the use of 2-methyl-1, 4-naphthoquinone in prothrombin deficiency. In daily doses of 1 to 4 mg. orally only one of ten patients failed to respond and no toxic effects were encountered.

*The curative chick unit of vitamin K (J. Biol. Chem. 123: CXXI, 1938) may be described as the smallest quantity of a vitamin K preparation given over a three-day period which will produce an average coagulation time of ten minutes or less in at least 50 per cent of baby chicks which have been fed a vitamin K-free diet for the first fourteen to fifteen days. The unit corresponds to the activity of approximately 50 mg. of dry alfalfa.

TABLE II

EFFECTIVENESS OF 2-METHYL-1, 4-NAPHTHOQUINONE IN HYPOPROTHROMBINEMIA OF OBSTRUCTIVE JAUNDICE WHEN DISSOLVED IN OIL AND GIVEN ORALLY
(Cases 101, 2Yo, 3Za, 4St, and 5Ba from Table I)

PATIENT NO.	PROTHROMBIN %		DAYS TREATED	DAILY DOSAGE (C.C.U.)
	BEFORE	AFTER		
101	30	80	3	2,400
3Za	38	90	6	2,400
4St	18	70	8	3,600
6Ti	28	90	3	3,600
7Bo	25	90	6	4,800
8Du	32	70	6	4,800
9Re	30	80	3	4,800
10Ha	28	70	10	4,800
11We	30	70	3	4,800
2Yo	35	65	7	7,200
5Ba	25	75	7	9,600

TABLE III

EFFECT OF 4-AMINO-2-METHYL-1-NAPHTHOL IN HYPOPROTHROMBINEMIA WHEN GIVEN INTRAVENOUSLY

PATIENT NO.	AGE	SEX	DIAGNOSIS	PROTHROMBIN %		DAYS TREATMENT	TOTAL DOSAGE VIT. K (MG.)	REMARKS
				BEFORE	AFTER			
1Mac	59 yr.	♀	Atrophic liver—C*	15	15	7	28	Died
2El	12 days	♂	Hemorrhagic disease of newborn	27	100	1	1	
3Tu	73 yr.	♂	Obstructive jaundice —C	40	70	11	22	
3Tu	73 yr.	♂	Second day post-operative	—	40	—	None	
3Tu	73 yr.	♂	Postoperative	40	80	14	28	Recovered
4Ga	25 yr.	♂	Catarrhal jaundice	50	90	4	8	
5Ev	39 yr.	♀	Cholecystitis with stone—T†	50	50	8	16	
6Ke	30 yr.	♂	Ulcerative colitis—C	50	50	11	44	No effect of vitamin K orally
7Wa	57 yr.	♀	Cholangeitis, chronic —T	28	60	3	6	
8St	62 yr.	♂	Hernia, strangulated —C	10	45	2	6	Died
9Ye	43 yr.	♂	Hernia, strangulated —C	18	45	6	12	
10St	51 yr.	♂	Obstructive jaundice —C	50	60	4	16	
11Ma	56 yr.	♀	Obstructive jaundice —C	5	80	6	12	
12Bu	3 days	♂	Hemorrhagic disease of newborn	No clot	80	1	6	
13Al	3 days	♀	Hemorrhagic disease of newborn	10	90	1	2	
14McF	31 yr.	♂	Gunshot wound liver —C	30	80	2	4	

*Confirmed.
†Tentative.

Given in doses of several milligrams daily for three to four weeks to numerous adult patients, 2-methyl-1, 4-naphthoquinone has developed no manifestations of intolerance. Rhoads and Fliegelman in their communication stated that they had given 0.5 mg. per kilogram of body weight without producing any

untoward phenomenon. It is the most potent of the oil-soluble vitamin K substances as judged by limited clinical experience.

Parenteral 4-Amino-2-Methyl-1-Naphthol Hydrochloride.—Further investigation of chemical substances having antihemorrhagic properties of vitamin K was continued in an effort to find a water-soluble and stable compound for parenteral use. This research resulted in the synthesis of 4-amino-2-methyl-1-naphthol and 4-amino-3-methyl-1-naphthol[17] as the hydrochloride, both of which are water soluble and stable. It was found, however, that the former possessed definitely greater vitamin K activity by the curative chick method than the latter—actually almost three times greater than K_1.[18] In Table III are given data on the use of 4-amino-2-methyl-1-naphthol hydrochloride in hypoprothrombinemia in man. Daily doses ranging from 1 mg. to 6 mg. (1,800 to 10,800 curative chick units in the form of the hydrochloride) given intravenously restored to normal severe prothrombin deficiency of obstructive jaundice within two to three days, while hemorrhagic states of the newborn responded within twelve hours to a dosage of 1 mg. intravenously (1,800 units).

DISCUSSION

Warner, Brinkhous, and Smith[19] have reported extensive observations on the antihemorrhage effect of vitamin K in obstructive jaundice, as have Butt, Snell, and Osterberg.[20] Hellman and Shettles,[21] and Waddell and Guerry[22] have studied hypoprothrombinemia in expectant mothers and in the newborn. Hellman and Shettles, using vitamin K concentrate, demonstrated that the plasma prothrombin of the newborn can be raised by administration to the mother prior to delivery and by direct use in the infant. Waddell and Guerry also demonstrated therapeutic effectiveness of vitamin K concentrate and 2-methyl-1, 4-naphthoquinone on prothrombin deficiency in the newborn, and that prenatal administration may prevent intracranial hemorrhage associated with normal delivery.

Our unpublished observations on several hundred adult patients show that depleted prothrombin is not uncommon in blood disorders associated with or without jaundice. Hypoprothrombinemia in some of these conditions has responded to vitamin K, but a complete evaluation must be deferred.

The isolation and synthesis of vitamins K_1 and K_2 constitute an epochal chemical achievement. Projection of chemical research beyond this phase, however, promises therapeutic affluence within the partially defined limits of usefulness of antihemorrhagic factors.

The effectiveness of parenteral 4-amino-2-methyl-1-naphthol, the absence of signs of intolerance, and its rapidity of action imply that prevention of hypoprothrombinemic bleeding in the newborn and adult will be a practical procedure. Further, that the control of bleeding associated with jaundice when surgical measures are urgent can be expected by the use of potent synthetic vitamin K substances by either the oral or the parenteral route of administration.

SUMMARY

A brief description of the chemical status of vitamin K_1 and synthetic substitutes having similar antihemorrhagic activity is given.

Seven patients having hypoprothrombinemia were ineffectively treated orally with 2-methyl-1, 4-naphthoquinone in dry form.

Five of the first series of seven patients and an additional six patients having prothrombin deficiency were successfully treated orally with 2-methyl-1, 4-naphthoquinone when the substance was dissolved in oil. No toxic effects were detected.

A water-soluble substance, 4-amino-2-methyl-1-naphthol hydrochloride, when given intravenously in hypoprothrombinemia, was effective in a majority of fourteen patients. Hemorrhagic tendency of the newborn responded rapidly. It has not produced toxic manifestations in the dosage used.

REFERENCES

1. Quick, A. J.: The Nature of the Bleeding in Jaundice, J. A. M. A. **110**: 1658, 1938.
2. Dam, H.: (a) Cholesterinstoffwechsel in Hühnereiern und Hühnchen, Biochem. Ztschr. **215**: 475, 1929; (b) Über die Cholesterinsynthese in Tierkörper, Biochem. Ztschr. **220**: 158, 1930.
3. Almquist, H. J., and Stokstad, E. L. R.: Dietary Hemorrhagic Disease in Chicks, Nature **136**: 31, 1935.
4. Almquist, H. J., and Stokstad, E. L. R.: Hemorrhagic Chick Disease of Dietary Origin, J. Biol. Chem. **111**: 105, 1935.
5. Doisy, E. A., Binkley, S. B., Thayer, S. A., and McKee, R. W.: Vitamin K, Science **91**: 58, 1940.
6. McKee, R. W., Binkley, S. B., Thayer, S. A., MacCorquodale, D. W., and Doisy, E. A.: The Isolation of Vitamin K_2, J. Biol. Chem. **131**: 327, 1939.
7. Binkley, S. B., MacCorquodale, D. W., Thayer, S. A., and Doisy, E. A.: The Isolation of Vitamin K_1, J. Biol. Chem. **130**: 219, 1939.
8. Almquist, H. J., and Klose, A. A.: Color Reactions in Vitamin K Concentrates, J. Am. Chem. Soc. **61**: 1611, 1939.
9. Anderson, R. J., and Newman, M. S.: Isolation of a Pigment and of Anisic Acid From the Acetone-Soluble Fat of the Human Tubercle Bacillus, J. Biol. Chem. **101**: 773, 1933.
10. Almquist, H. J., and Klose, A. A.: The Antihemorrhagic Activity of Certain Naphthoquinones, J. Am. Chem. Soc. **61**: 1923, 1939.
11. McKee, R. W., Binkley, S. B., MacCorquodale, D. W., Thayer, S. A., and Doisy, E. A.: The Isolation of Vitamins K_1 and K_2, J. Am. Chem. Soc. **61**: 1295, 1939.
12. MacCorquodale, D. W., Binkley, S. B., Thayer, S. A., and Doisy, E. A.: On the Constitution of Vitamin K_1, J. Am. Chem. Soc. **61**: 1928, 1939.
13. Ansbacher, S., and Fernholz, E.: Simple Compounds With Vitamin K Activity, J. Am. Chem. Soc. **61**: 1924, 1939.
14. Fries, K., and Lohmann, W.: Untersuchungen in der Reihe des 2-Methyl-Naphthaline, Ber. Chem. Ges. **54**: 2912, 1921.
15. Sharp, E. A.: Vitamin K Activity of 2-Methyl-1, 4-Naphthoquinone, Proc. Cen. Soc. Clin. Res. J. A. M. A. **114**: 439, 1940.
16. Rhoads, J. E., and Fliegelman, M. T.: The Use of 2-Methyl-1, 4-Naphthoquinone (A Synthetic Vitamin K Substitute) in the Treatment of Prothrombin Deficiency in Patients, J. A. M. A. **114**: 400, 1940.
17. Doisy, E. A., MacCorquodale, D. W., Thayer, S. A., Binkley, S. B., and McKee, R. W.: The Isolation, Constitution and Synthesis of Vitamin K_1, Science **90**: 407, 1939.
18. Emmett, A. D., Brown, R. A., and Kamm, O.: Comparison of the Antihemorrhagic Activity of Natural and Synthetic Vitamin K_1 With the Proposed Standard 2-Methyl-1, 4-Naphthoquinone, J. Biol. Chem. **132**: 467, 1940.
19. Warner, E. D., Brinkhous, K. M., and Smith, H. P.: Bleeding Tendency of Obstructive Jaundice: Prothrombin Deficiency and Dietary Factors, Proc. Soc. Exper. Biol. & Med. **37**: 628, 1938.
20. Butt, H. R., Snell, A. M., and Osterberg, A. E.: (a) The Use of Vitamin K and Bile in Treatment of the Hemorrhagic Diathesis in Cases of Jaundice, Proc. Staff Meet., Mayo Clin. **13**: 74, 1938; (b) Snell, A. M., Butt, H. R., and Osterberg, A. E.: Treatment of the Hemorrhagic Tendency in Jaundice, With Special Reference to Vitamin K, Am. J. Digest. Dis. & Nutrition **5**: 590, 1938.
21. Hellman, L. M., and Shettles, L. B.: Factors Influencing Plasma Prothrombin in the Newborn Infant, Bull. Johns Hopkins Hosp. **65**: 138, 1939.
22. Waddell, W. W., Jr., and Guerry, D., III: The Role of Vitamin K in the Etiology, Prevention and Treatment of Hemorrhage in the Newborn Infant, J. Pediat. **15**: 802, 1939.

MEAT EXTRACTIVES IN STUDIES OF GASTRIC FUNCTION*

R. S. Fisher, B.S., and Frank L. Apperly, M.D., Richmond, Va.

IT HAS long been recognized that meat extractives increase gastric secretion, but until recently there have appeared few data on the quantitative measurement of this response. Tessieri (1930)[6] stated that ingestion of peptone preparations increased the duration and quantity of human gastric secretion. Komm (1931)[4] reported that "Tago," a commercial meat hydrolysate, caused a marked increase in the gastric secretion of dogs. Smith and Cowgill (1933)[5] reported the results of preliminary studies on gastric secretion following administration of various meat extracts. Wilhelmj, O'Brien, and Hill (1936)[8] advocated a test meal of meat extractives and described its effect on gastric secretion in dogs.

The most important work of a quantitative nature which has been done on human beings appeared in 1939, when Upham and Spindler,[7] using the test meal as evolved by Wilhelmj and associates, reported their studies of the response of the normal and of certain pathologic stomachs to the meat extractive secretagogue meal. This test meal was prepared by a rather lengthy and laborious treatment of a commercially available meat paste.

Meat extracts have been marketed for over sixty years and are prescribed in certain types of gastrointestinal dysfunction. Little is known, however, of the mechanism involved in the beneficial effects resulting from this therapy. This study was undertaken, therefore, with the dual purpose of obtaining quantitative data on the secretagogue power of a commercially available meat extract which would require little or no chemical treatment before administration as a test meal and of gaining information concerning the gastric response to the administration of a meat extract with the hope of explaining to some extent the mechanism of its action.

PROCEDURE

The test subjects used were healthy male medical students, acquainted with the use of the Rehfuss tube and the purposes of the investigation. The nature of the meals used, the lack of discomfort during the test period, and the reversal of the order of administration of test meal and control meal in one-half of the persons minimized and neutralized any possible psychic disturbances of gastric secretion. In fact, in individuals who were used repeatedly for the same test meal, the striking similarity of the secretory responses (noted by other observers) showed that our results were valid for comparative purposes.

The technique followed the usual procedure, viz., the introduction of the Rehfuss tube, aspiration of fasting contents after a rest of fifteen minutes, and

*From the Departments of Biochemistry and Pathology, Medical College of Virginia.
Received for publication, February 5, 1940.

the introduction of the previously warmed fluid meal via the tube. Samples were removed at fifteen minutes, thirty minutes, and at thirty-minute intervals thereafter. Care was taken to mix the stomach contents well before withdrawing each sample.

The meals used were: (a) The control meal, prepared by adding 25 Gm. of Ralston (a wheat cereal) to 750 c.c. of boiling water, boiling with stirring for ten minutes, allowing to stand for several hours and decanting 400 c.c. of supernatant fluid. To this was added 1 c.c. of phenol red (6 mg. per cubic centimeter, Hynson, Westcott, and Dunning). This preparation was cloudy owing to suspended starchy material. (b) The meat extract meal (used for series I) was prepared by the addition of 16 c.c. of meat extract,* neutralized to Töpfer's reagent with 5 N hydrochloric acid, to sufficient gruel (prepared as above) to make a total volume of 400 c.c.

Free acidity of the samples withdrawn from the stomach was determined by dilution and titration with fresh 0.01 N sodium hydroxide solution, using Töpfer's reagent as indicator; total chlorides were determined by the Van Slyke and Sendroy modification of Volhard's method; pepsin was determined by Mett's method. The amount of dilution of the gastric contents by various secretions was determined in the samples by comparison in a microcolorimeter of the color of the phenol red in the test meal (after alkalinization) with that of the standard meal used (also after alkalinization to bring out the color).

In series II and III where meat extract, diluted with water instead of gruel meal, was used, it was possible to determine the dilution with fair accuracy without any treatment other than the addition of alkali. Sometimes the brownish color of the standard was matched by adding minute amounts of meat juice to the unknown. Samples containing bile were matched by adding a trace of picramic acid to the standard. Where the suspended starchy material in the gruel meals interfered with color comparison, it was removed before alkalinization by suction filtration through asbestos mats. In most cases, however, an accuracy of 2.5 per cent could be obtained without such treatment, and it was felt that in laboratory practice the loss of time required to attain further accuracy would not be justified.

RESULTS

Fig. 1 shows the curves obtained by averaging the values for all samples at corresponding intervals in ten pairs of test meals. In plotting the total chloride curve for the meat extract meal, the analytical values obtained on the samples were first corrected for the chlorides contained in the test meal before ingestion (due to salt in the meat extract and to chlorides added as hydrochloric acid in neutralization to Töpfer's reagent). The following formula was used:

$$Cl_{int.} = A - A_o X.$$

A represents the chloride concentration of the sample analyzed; X, the per cent of indicator present in the sample analyzed; and A_o, the chloride concentration of the original test meal. (A_o for the meal used in this investigation was 47 c.c. of 0.1 normal chloride per 100 c.c. of test meal.) Actually in in-

*The meat extract used in this investigation was kindly donated by the Valentine's Meat Juice Co., Richmond, Va.

dividual test meals somewhat higher peak values were often observed, but their distribution in the samples of different time intervals resulted in the average values shown.

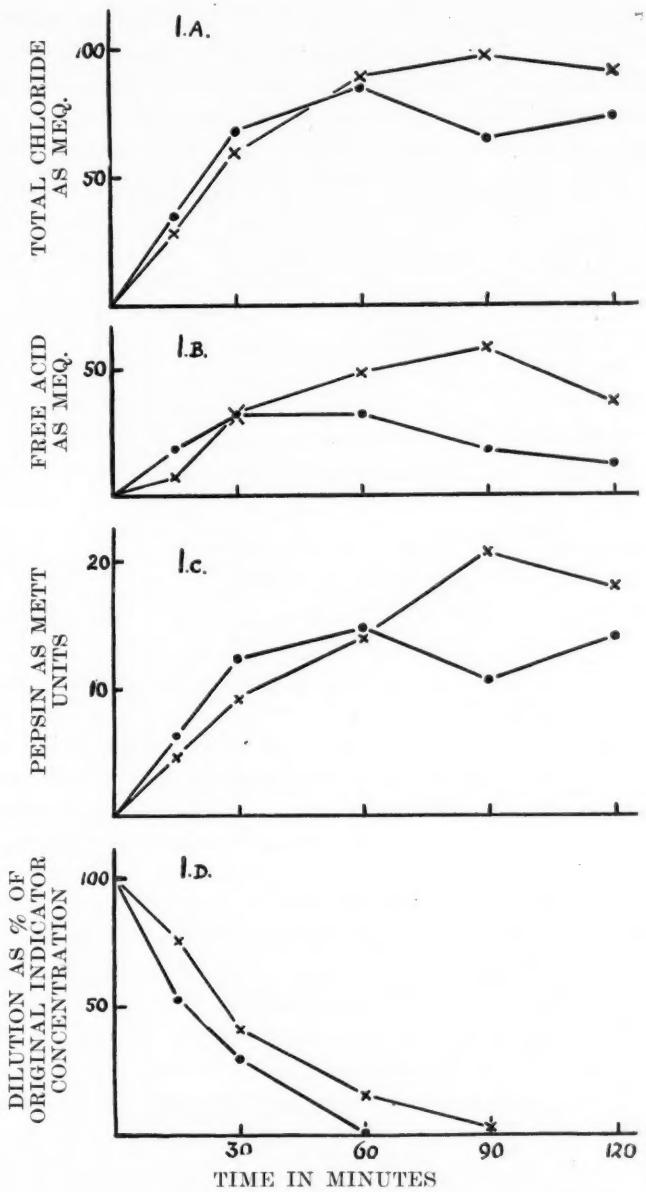


Fig. 1.—×× Gruel plus meat juice extract. • • Gruel meal.

As Fig. 1 shows, in the meat extract meal, and especially in the second hour, there is a higher concentration of total chloride, free acid, and pepsin. These differences occur only after the stomachs have emptied. This state of affairs might have been brought about by one of several things:

1. A greater secretion of acid gastric juice into the *meat extract* meal. This would also lead to a greater dilution of the meat extract meal than of the gruel meal, whereas actually the reverse has occurred (Fig. 1 *D*).

2. A greater secretion into the *gruel meal* of a chloride solution containing less hydrochloric acid than that secreted in the first hour. Since, however, in gastric juice of varying hydrochloric acid content the hydrochloric acid plus sodium chloride equals a constant of about 0.170 normal (Hollander),[2] the total chloride of the gastric secretion must remain unchanged. On the contrary Fig. 1 *A* shows that in the case of the gruel meal the total chloride diminished rapidly in the second hour.

3. A regurgitation of duodenal fluids containing chloride and bicarbonate into the gruel meal. This possibility is the only one that can account for all the phenomena found, i.e., the lower total chloride, the relatively still lower free acid, the lower pepsin, and the greater dilution of stomach contents.

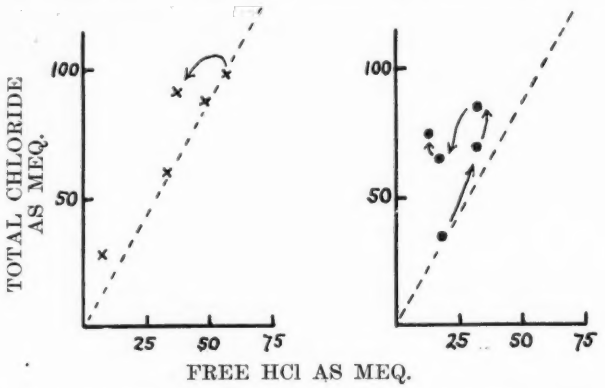


Fig. 2.—×× Gruel plus meat juice extract. • • Gruel meal.

In Fig. 2 these results, at least as regards the relationship of total chlorides to free acid, have been compared in another way, by plotting the latter against the former. Hollander (1932)[2,3] has shown that when various samples of pure gastric juice are drawn from animals, the plot of total chlorides against the corresponding free acid yields a straight line relationship. In our gruel and meat extract meal, where the gastric chloride and acid are diluted only by the fluid meal during the first hour, a similar straight line relationship exists; in the second hour other factors begin to operate, with fall of acidity. In the gruel meal, however, the free acidity very soon falls to the left of the straight line, showing that alkalies (presumably of duodenal origin) are neutralizing the free acid only half an hour after the meal was placed in the stomach.

Apperly and Semmens (1928)[1] showed that high total chlorides, high acidity, and increased emptying time in healthy stomachs, indicate a higher gastric muscle tonus. They further showed that the duodenum is not so readily able to regurgitate its contents into a hypertonic, relatively contracted stomach. This results in a higher acidity of the stomach contents, which in turn, as is well known, delays the emptying time, as judged by the disappearance of food, starch, or dye from the stomach. These are the conditions found in the meat extract meal, which would, therefore, appear to be directly or indirectly responsible for the increased tonicity of the stomach. Clinically it is well known that the latter manifests itself in increased appetite and sense of well-being, and thus probably accounts for the popularity of meat extracts during con-

valescence and poor health. The increased concentration of acid and pepsin and the slower emptying time would also increase the rate and degree of digestion of certain foods.

A further series of test meals (series II) was prepared by dilution of 16 c.c. of meat extract to 399 c.c. with distilled water, and the addition of 1 c.c. of the phenol red solution as in the other test meals, but the results were not significantly different from those in series I. The peak acidity and pepsin values were reached sooner, but not as soon as with the gruel meal alone. Their values were approximately the same. Emptying time also was less than with the combination meal, but showed a delay when compared with the gruel meal.

A third (series III), using a 1:50 dilution of the meat juice, was done in order to study the effect of greater dilution of the meat extractives, the results being similar to those in series II, except that the peak values for concentration of secreted substances were reduced, indicating that the maximum effect of the meat extract was not being obtained. Since use of diluted meat extract alone produces results similar to those found with gruel plus meat extract, the use of meat extract alone as a test meal is advocated. Further work on this test meal is contemplated.

SUMMARY

An examination of the mode of action of a commercially obtainable meat extract was made in order to ascertain the physical basis, if any, for the commonly alleged sense of well-being accompanying the use of such extracts.

In comparison with the ordinary test meal alone, the addition of meat extract to the meal raises the concentration of total chlorides, free acid, and pepsin, and retards the dilution of the gastric contents. These facts are interpreted as indicating an increased tonicity of gastric muscle, which is known to be the physical basis of appetite.

Our experience with meat extract leads us to prefer this substance to all others for routine and experimental test meal work.

We wish to express our appreciation to Dr. J. C. Forbes for his helpful suggestions during the course of this work.

REFERENCES

1. Apperly, F. L., and Semmens, K. M.: Variations in Normal Gastric Functions and Their Causes. Some New Experimental and Clinical Observations, M. J. Australia **2**: 226, 1928.
2. Hollander, F.: Studies in Gastric Secretion. IV. Variations in the Chlorine Content of Gastric Juice and Their Significance, J. Biol. Chem. **97**: 585, 1932.
3. Hollander, F., and Cowgill, G. R.: Studies in Gastric Secretion. I. Gastric Juice of Constant Acidity, J. Biol. Chem. **91**: 151, 1931.
4. Komm, E.: Über die Einwirkung von Fleischhydrolysaten auf die Magensaftabsonderung, Klin. Wehnschr. **10**: 981, 1931.
5. Smith, E. R. B., and Cowgill, G. R.: Meat Extractives as Stimulants for Secretion of Pepsin, Proc. Soc. Exper. Biol. & Med. **30**: 1284, 1933.
6. Tessieri, Iris: The Action of Peptone on the Gastric Secretion, Semana méd. **37**: 1579, 1930.
7. Upham, Roy, and Spindler, F.: Studies on Humans With a New Secretagogue Meal, Am. J. Digest. Dis. **5**: 721, 1939.
8. Wilhelmj, C. M., O'Brien, F. T., and Hill, F. C.: An Improved Gastric Test Meal, Am. J. Digest. Dis. & Nutrition **3**: 319, 1936.

FURTHER OBSERVATIONS ON THE ROLE OF DIET IN THE ETIOLOGY AND TREATMENT OF SPONTANEOUS HYPOGLYCEMIA*

L. W. Swanson, M.D., and James A. Greene, M.D., Iowa City, Iowa

THE manifestations of spontaneous hypoglycemia are sufficiently well recognized to require no further comment. There are apparently several causes for the syndrome, and once the diagnosis has been definitely established proper therapy cannot be instituted until the etiology is ascertained. It was assumed at first that all cases were due to an overproduction of insulin by the pancreas. This assumption led to the frequent feeding of carbohydrates to the milder cases and to surgical exploration for pancreatic tumor in the severe ones. It soon became apparent that pancreatic tumors were not present even in all of the extreme cases and that other etiologic factors must be considered. Faulty dietary habits appeared to be a likely etiologic factor in certain cases, and such a concept was supported by the fact that many patients became worse when treated with a high carbohydrate diet divided into several small meals daily. A low carbohydrate, high fat diet was proposed, therefore, as a therapeutic procedure by Clark and Greene,[1] and by Waters,[2] with successful results. Conn[3] proposed a diet high in fat and protein, and low in carbohydrates after he observed a patient who had become worse with a high carbohydrate diet and did not improve with a high fat diet. He demonstrated that there was no appreciable hyperglycemia following such a diet and, therefore, there was no hypoglycemia. His patient was relieved of manifestations for a period of three months. Newburgh and Conn[4] then observed six patients with spontaneous hypoglycemia and infectious hepatitis which was usually secondary to gall bladder disease. A cure was obtained in one case following removal of a diseased gall bladder. They attributed the disturbance of carbohydrate metabolism to the hepatitis.

Since the report of Clark and Greene,[1] we have observed eleven patients with spontaneous hypoglycemia due to faulty dietary habits. It was thought at first that all such cases were due to an excess carbohydrate intake until one patient who was observed in the Out-Clinic where the diagnosis was established, was admitted to the ward and given a high carbohydrate diet in an attempt to make the condition worse. On the contrary, the symptoms subsided and the glucose tolerance returned to normal. A careful dietary history then revealed that he had followed a high fat and low carbohydrate diet for several months prior to and after development of the spontaneous hypoglycemia. Since then we have

*From the Department of Internal Medicine State University of Iowa, College of Medicine, Iowa City.

Received for publication, January 30, 1940.

observed two other patients who have developed their symptoms while on a high fat and low carbohydrate diet. The remaining patients have followed a high carbohydrate and low fat type of diet. The two cases here presented are typical illustrations of the two types of faulty diets which will produce spontaneous hypoglycemia. Case 1 illustrates the high carbohydrate and low fat diet as an etiologic factor, and Case 2 illustrates the low carbohydrate and high fat dietary type.

CASE 1.—A male dry cleaner, aged 32 years, entered the University Hospitals in July, 1937, because of a stone in the right lower ureter. His symptoms disappeared after spontaneous passage of the stone, but he returned in March, 1938, complaining of vague abdominal pain, constipation, palpitation, and nervousness. No organic disease was discovered and he was sent home without treatment.

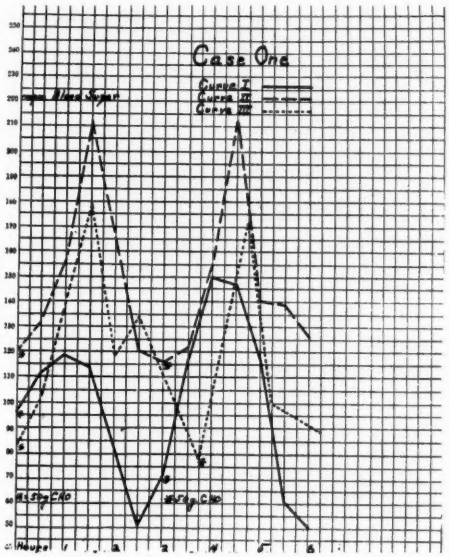


FIG. 1.—The eradication of hypoglycemia in Case 1 by a diet high in fat and low in carbohydrate, as shown by the double glucose tolerance curves.

He returned again in September, 1938, however, complaining of increasing nervousness and irritability, weakness, hunger, palpitation, fatigability, insomnia, mild constipation, slight abdominal distress, and loss of five pounds of body weight in spite of an excellent appetite. Most of these symptoms occurred two or three hours after each meal, and were severe enough to force him to stop work. Food relieved the symptoms at such times sufficiently for him to resume work, and hence he habitually ate between meals. A bedtime lunch would also prevent the symptoms at night. His diet had been extremely high in carbohydrate and low in fat, primarily because of a dislike for fatty foods.

The physical examination revealed no noteworthy abnormalities. The double glucose tolerance curve (curve I, Fig. 1) was typical for hypoglycemia. After six days of a diet containing 70 Gm. of protein, 116 Gm. of carbohydrate, and 211 Gm. of fat, the double glucose tolerance curve (curve II, Fig. 1) was within normal limits and the symptoms had disappeared completely. He was discharged with a diet containing 80 Gm. of protein, 170 Gm. of carbohydrate, and 200 Gm. of fat.

He returned four months later entirely free of symptoms, and the glucose tolerance curve (curve III, Fig. 1) continued within normal limits. He was sent home with instructions to follow a diet containing 125 Gm. of protein, 200 Gm. of carbohydrate, and 250

Gm. of fat, and to return to the clinic if the symptoms reappeared. A report one year later stated that he was entirely free of symptoms.

CASE 2.—A farmer, aged 28 years, entered the University Hospitals in 1938, because of abdominal distress, nervousness, palpitation, and sweating. He had enjoyed good health until 1936, when alternating constipation and diarrhea, heartburn, water brash, vomiting, and slight generalized abdominal distress appeared. He was examined by his local physician who prescribed a bland diet. In addition, because of a dislike for carbohydrate foods, the patient reduced the carbohydrate content of his diet to a minimum. The symptoms disappeared, but several months later they returned and were accompanied by weakness, palpitation, and sweating. These symptoms occurred two to three hours after meals, and it was soon discovered that eating between meals would give relief until the next meal. The symptoms increased, however, and became so severe that he had to stop work.

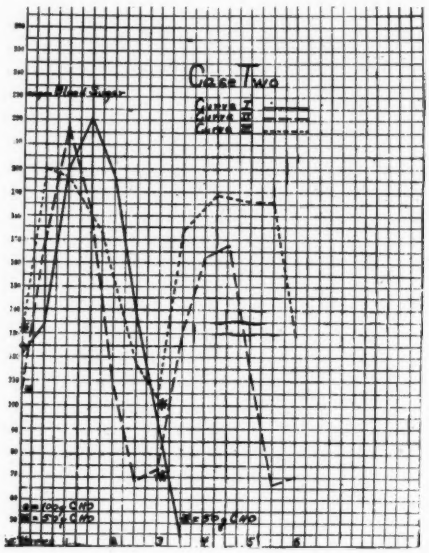


Fig. 2.—Hypoglycemia disappeared in Case 2 following a diet high in carbohydrate and low in fat, as shown by the double glucose tolerance curves.

The physical examination revealed no noteworthy abnormalities. The gastroenteric tract was normal to roentgen-ray studies. The glucose tolerance curve (curve I, Fig. 2) was typical of hypoglycemia. The patient was sent home for three weeks with instructions to follow a diet containing 500 Gm. of carbohydrate daily. He did not follow the instructions completely, but the symptoms were less when he returned and the double glucose tolerance curve (curve II, Fig. 2) was improved. He was discharged with the advice to follow the instructions and to return in three months. He returned two and one-half months later, stating that he had followed the diet, that his symptoms had completely disappeared, and that he had resumed work. The double glucose tolerance curve (curve III, Fig 2) was greatly improved. He was instructed regarding a diet containing 90 Gm. of protein, 350 Gm. of carbohydrate, and 175 Gm. of fat before discharge and advised to return if his symptoms reappeared. A report one year later stated that he was entirely well.

COMMENT

There appear to be several causes for spontaneous hypoglycemia. Once the diagnosis is established the etiology must be ascertained before proper treatment can be instituted. The known causes for spontaneous hypoglycemia are (1) an oversecretion of insulin due to either tumor of the pancreas or hyperplasia of the

islets of Langerhans; (2) Addison's disease; (3) probably certain pituitary dysfunctions; (4) infectious hepatitis, which is usually secondary to gall bladder disease; and (5) faulty dietary habits. The syndrome can be produced by either extreme in the carbohydrate and fat content of the diet: A high carbohydrate and low fat diet or a low carbohydrate and high fat diet can produce the manifestations.

It is usually not difficult to establish the etiology in a given case. The manifestations in patients with an oversecretion of insulin are usually extreme and are not improved by dietary therapy, although they may be partially or entirely alleviated as long as adequate and frequent carbohydrate intake is continued. Addison's disease is not likely to be overlooked if its presence is once considered. The presence of pituitary dysfunction can be ascertained only when there are other manifestations of such dysfunction, and these cases are undoubtedly extremely rare. An infectious hepatitis may produce severe manifestations, and these cases are probably more common than the above-mentioned ones. A thorough study of the biliary tract is indicated in each case of spontaneous hypoglycemia, but the absence of evidence of gall bladder disease does not absolutely exclude hepatitis, nor does the presence of gall bladder disease definitely establish its presence. In patients with spontaneous hypoglycemia due to faulty dietary habits the manifestations are usually mild, but occasionally they are severe. There are many functional complaints in patients with spontaneous hypoglycemia due to faulty diets, and these complaints frequently obscure those of hypoglycemia. In fact, the functional complaints have caused most of these patients to follow the abnormal diet. The eradication of the hypoglycemic manifestations does not cure such patients, but the neurotic tendencies cannot be controlled as long as the symptoms of hypoglycemia persist. A careful dietary history is necessary, therefore, in such cases to ascertain whether or not the previous diet has been high in fat or high in carbohydrate. A high carbohydrate diet will not eradicate hypoglycemia if this type of diet has produced it, but a high fat diet will correct it. The reverse is true if a high fat diet produced the syndrome.

If the manifestations are extreme, the etiology of a given case of spontaneous hypoglycemia is most likely an infectious hepatitis or an overproduction of insulin from a pancreatic tumor. The former etiologic factor can be ascertained fairly accurately by a careful study of the biliary tract. On the other hand, the etiology is most likely an infectious hepatitis or a faulty diet if the manifestations are mild or moderately severe. The latter, in our opinion, is the most common cause for spontaneous hypoglycemia and can be uncovered by a careful dietary history and by the response to a diet which contains carbohydrate and fat in the reverse proportion to that which the patient has followed previously.

The time required for such diets to eradicate hypoglycemia in these patients varies considerably. It has required from six days to six months in our patients. The minimum duration for such dietary regimen after correction of the hypoglycemia is not known. We have assumed that the patients who respond quickly need not follow such a diet as long after correction of the hypoglycemia as those who are slower to respond. We, therefore, have advised our patients to

adhere to such diets for periods varying from three weeks to two and one-half years after the hypoglycemic manifestations have subsided. Probably such patients should be re-examined at three- to six-month intervals during therapy.

It is important that the patient be informed of the cause of the manifestations and that adequate general dietary instructions be given which will prevent a repetition of dietary mistakes after the specific dietary regime has been discontinued.

SUMMARY

The causes of spontaneous hypoglycemia have been discussed and those due to faulty diets have been emphasized. The syndrome can be produced by diets low in carbohydrate and high in fat, or by those high in carbohydrate and low in fat. Three cases due to the former and eight due to the latter have been observed, but only one case illustrative of each dietary etiology is reported. The types of corrective diets and the duration of therapy have been discussed.

REFERENCES

1. Clark, B. B., and Greene, J. A.: Effect of Low Carbohydrate Diet on Glucose Tolerance in Spontaneous Hypoglycemia, Proc. Soc. Exper. Biol. & Med. **32**: 1459, 1935.
2. Waters, W. C., Jr.: Spontaneous Hypoglycemia: The Role of Diet in Etiology and Treatment, South. Med. J. **24**: 249, 1931.
3. Conn, J. W.: The Advantage of a High Protein Diet in the Treatment of Spontaneous Hypoglycemia, J. Clin. Investigation **15**: 673, 1936.
4. Conn, J. W., Newburgh, L. H., Johnston, M. W., and Sheldon, J. M.: Study of the Deranged Carbohydrate Metabolism in Chronic Infectious Hepatitis, Arch. Int. Med. **62**: 765, 1938.

THE EFFECT OF COPPER AND IRON ON HEMOGLOBIN REGENERATION*

WILLIS M. FOWLER, M.D., AND ADELAIDE P. BARER, PH.D.
IOWA CITY, IOWA

THAT copper plays a role in the synthesis of hemoglobin has been conclusively demonstrated, but since it is not a part of the hemoglobin molecule, the exact mechanism of its action has not been ascertained, and the clinical implications to be drawn from animal experimentation are not entirely clear. The numerous reports concerning its effect on the anemia of experimental animals have been adequately summarized.[1-5] The present concept, in brief, is that copper acts as a catalytic agent in hemoglobin formation and aids in the mobilization and utilization of iron stored in the body as well as augmenting the effect of inorganic iron salts administered by mouth. These effects are illustrated by the fact that neither iron nor copper alone are effective in the treatment of nutritional anemia in the rat, whereas a combination of the two produces a rapid hemoglobin response. When iron has been given to an anemic rat without producing a hemoglobin response and its administration then discontinued, it has been found that copper alone will then produce rapid hemoglobin regen-

*From the Department of Internal Medicine, State University of Iowa College of Medicine, Iowa City.
Received for publication, February 21, 1940.

eration by aiding in the utilization of the iron previously stored.[3] The results in chronic anemia in dogs have been similar but less consistent than those observed in rats.[6, 7]

The clinical reports on the use of copper and iron in the treatment of anemia are conflicting. An increase in the hemoglobin of infants has been reported following the use of 25 mg. of iron and 1 mg. of copper daily, and in severe nutritional anemia the hemoglobin response has been rapid. When the same amount of iron was administered alone, the response was less satisfactory.[8, 9] This combination of copper and iron not only has been more effective than iron alone in raising the hemoglobin level but also has caused a greater gain in weight and lessened the incidence of infections among infants.[10] In adults the administration of copper and iron has been reported to be effective in "secondary anemia,"[11] idiopathic hypochromic anemia,[12] and in anemia which accompanies the therapeutic use of malaria.[13] Other reports have indicated that the addition of copper to an inorganic iron salt was not necessary,[14] and in a study of a group of college women iron alone produced as good results as did the combination of iron and copper.[15] It has been assumed in the cases in which iron alone has produced a satisfactory response that either there was no copper deficiency or there was sufficient copper obtained from the food or as a contaminant of the inorganic iron salt.

In a previous report[16] concerning the effect of copper on iron metabolism in adult patients with hypochromic anemia, it was shown that the addition of copper sulfate to a relatively small dose of iron and ammonium citrates (217 to 261 mg. of elemental iron per day) led to a diminution in the amount of iron retained by the body but that hemoglobin regeneration was active in spite of the diminished iron retention. In two other patients receiving larger amounts of iron and ammonium citrates (426 and 517 mg. of iron per day) the addition of copper sulfate did not influence the retention of iron. In both groups of patients hemoglobin regeneration was active regardless of the amount of iron retained, but in neither group was the increase more rapid than that obtained with iron alone.

In an attempt to gain further information on the effect of copper on hemoglobin formation, 20 patients with mild grades of hypochromic anemia were selected for study. All were females and all were nurses, students, or hospital employees who would cooperate and who could be depended upon to take the medication regularly. One patient was found to have menorrhagia and was dropped from the study, and others dropped out after the first and second periods. The study was divided into three periods, each of six weeks' duration. During the first period the patients received 0.5 Gm. (7.5 grains) of iron and ammonium citrates daily. During the second period they received the same amount of iron plus copper sulfate, and during the third period the copper was discontinued but the dose of iron and ammonium citrates was increased to 1 Gm. daily. Hemoglobin determinations by the Newcomer method, erythrocyte counts, and hematocrit determinations were made at approximately two-week intervals on all patients. Except for the initial readings, only the hemoglobin values are recorded in this communication, since these present the essential information. All medications were supplied in gelatin capsules.

RESULTS

Period I. Iron and ammonium citrates 0.5 Gm. per day (approximately 85 mg. of elemental iron). At the onset of therapy the blood hemoglobin values ranged from 8.46 to 11.57 Gm. per 100 c.c., with an average of 9.88 Gm. for the 19 patients. Following the administration of iron for forty-two days, the average gain in hemoglobin per patient was 0.41 Gm. The gain or loss for each patient is shown in Table I. The greatest increase in hemoglobin was

TABLE I

INITIAL BLOOD VALUES AND HEMOGLOBIN READING AT THE END OF EACH OF THREE PERIODS WITH INCREASE OR DECREASE DURING THAT PERIOD

CASE	INITIAL BLOOD VALUES			PERIOD I		PERIOD II		PERIOD III	
	R.B.C. MILLIONS	HEMAT. %	HB. GM.	HB. GM.	INCREASE GM.	HB. GM.	INCREASE GM.	HB. GM.	INCREASE GM.
1	4.05	34	9.32	10.07	+0.75				
2	4.19	33	9.62	9.62	0				
3	4.34	34	10.37	10.52	+0.15				
4	4.88	34	8.46	9.02	+0.56				
5	4.17	37	10.37	10.94	+0.57	10.94	0		
6	3.61	34	9.47	9.92	+0.45	9.32	−0.60		
7	4.91	40	11.57	11.15	−0.42	11.57	+0.42		
8	4.44	35	11.36	11.78	+0.42	11.99	+0.21		
9	4.35	37	10.52	10.73	+0.21	11.15	+0.42		
10	3.62	34	8.90	10.07	+1.17	10.37	+0.30		
11	4.07	36	10.37	11.57	+1.20	11.36	−0.21	10.52	−0.84
12	4.08	35	10.52	10.73	+0.21	10.94	+0.21	10.94	0
13	3.65	35	10.22	10.22	0	10.07	−0.15	11.15	+1.08
14	3.95	34	8.80	9.62	+0.82	10.37	+0.75	10.37	0
15	4.57	32	8.68	8.90	+0.22	9.77	+0.87	10.22	+0.45
16	3.67	35	9.92	9.92	0	10.52	+0.60	10.73	+0.21
17	3.82	37	10.22	10.07	−0.15	10.52	+0.45	10.94	+0.42
18	4.80	38	10.07	11.36	+1.29	10.94	−0.42	10.94	0
19	4.22	40	9.02	9.47	+0.45	10.07	+0.60	11.78	+1.71
Average gain					+0.415		+0.233		+0.336

found in Case 11, who had an initial hemoglobin value of 10.37 Gm. and during the six-week period showed a gain of 1.2 Gm. In 3 patients (Cases 2, 13, and 16) there was no hemoglobin increase, and in 2 patients (Cases 7 and 17) there was a decrease in spite of this therapy. This slow and inconsistent hemoglobin increase cannot be considered as a satisfactory response to an anti-anemic remedy, but this small dose of medicinal iron was purposely chosen so that the response would not be adequate. It was felt that if copper was of value in increasing the effectiveness of inorganic iron in hemoglobin formation, it would be manifest by an increased rate of regeneration when the copper was added to a small dose of iron. By using an amount of iron too small to be efficient, the beneficial effects of copper would not be masked by the rapid increase obtained with large doses of iron.

Period II. Iron and ammonium citrates 0.5 Gm. per day plus 3 mg. of copper as copper sulfate (ratio of Fe to Cu 28:1). Four patients discontinued treatment after the first period, leaving 15 under observation during the second period. The hemoglobin values ranged from 9.47 to 11.78 Gm., with an average of 10.43 for the 15 patients. During this period of six weeks the average gain in hemoglobin was 0.23 Gm. The greatest increase was in Case 15, with a gain of 0.87 Gm. In one patient (Case 5) there was no increase in hemoglobin, and

in 4 patients (Cases 6, 11, 13, and 18) there was a decrease. In 6 patients the hemoglobin increase was more rapid with copper and iron than it had been with iron alone; in one patient it was the same; but in the other 8 patients it gained more rapidly before the addition of copper. The average hemoglobin increase was lower than that obtained with the same amount of iron alone, so that the addition of copper did not appreciably increase the effectiveness of inorganic iron. The average hemoglobin level at the onset of treatment with copper and iron was slightly higher than when iron alone was started (10.43 Gm. as compared to 9.88 Gm.), but this is not great enough to make an appreciable difference.

Period III. Iron and ammonium citrates, 1 Gm. daily (approximately 170 mg. of elemental iron) without copper sulfate. Nine patients were followed through the third period and received twice the amount of iron previously administered. The blood hemoglobin values at the start of the period ranged from 9.77 to 11.36 mg., with an average of 10.50 Gm. The average gain in hemoglobin per patient for this six-week period was 0.34 Gm. The greatest gain was 1.71 Gm. in Case 19. Three patients failed to gain (Cases 12, 14, and 18), and one (Case 11) showed a decrease. The average hemoglobin increase was greater than that obtained with half the amount of iron plus copper sulfate and shows that the patients still have the ability to respond when iron is available in sufficient amounts.

It has been our experience with iron therapy that (in patients with mild anemias) a peak in hemoglobin regeneration is reached and thereafter there is a slight drop in the hemoglobin level even though the iron is continued. In this group of 9 patients such a peak was reached before the end of the period (in 6 instances), and a subsequent drop in hemoglobin occurred. Had the hemoglobin gain been calculated from the highest level rather than the reading at the end of six weeks, the average gain would have been 0.54 Gm. rather than 0.34 Gm.

It is possible that the greater hemoglobin increase during period III as compared to period II might be due to the copper which was stored in the body during the preceding six weeks rather than to the increased amount of iron administered. However, in another group of 12 patients with a similar hemoglobin level (10.32 Gm. per 100 c.c.) who were started immediately on 1 Gm. of iron and ammonium citrates per day, there was an average gain of 1.29 Gm. of hemoglobin during a six weeks' period. This is still greater than the gain recorded in the 9 patients during period III and indicates that the increased effect was due to the increased amount of iron rather than to the previously administered copper.

SUMMARY

The addition of copper sulfate to an inorganic iron salt did not increase the effectiveness of iron in hemoglobin regeneration in mild hypochromic anemias in the adult.

REFERENCES

1. Elvehjem, C. A.: The Biological Significance of Copper and Its Relation to Iron Metabolism, Physiol. Rev. **15**: 471, 1935.
2. Josephs, H. W.: Studies on Iron Metabolism and the Influence of Copper, J. Biol. Chem. **96**: 559, 1932.

3. Elvehjem, C. A., and Sherman, W. C.: The Action of Copper in Iron Metabolism, J. Biol. Chem. **98**: 309, 1932.
4. Cook, S. F., and Spilles, N. M.: Some Factors Regulating the Utilization of Splenic Iron, Am. J. Physiol. **98**: 626, 1931.
5. Elvehjem, C. A., and Hart E. B.: The Necessity of Copper as a Supplement to Iron for Hemoglobin Formation in the Pig, J. Biol. Chem. **95**: 363, 1932.
6. Robscheit-Robbins, F. S., Elden, C. A., Sperry, W. M., and Whipple, G. H.: Blood Regeneration in Severe Anemia. XII. Potent Influence of Inorganic Ash of Apricots, Liver, Kidney, and Pineapple, J. Biol. Chem. **79**: 563, 1928.
7. Elden, C. A., Sperry, W. M., Robscheit-Robbins, F. S., and Whipple, G. H.: Blood Regeneration in Severe Anemia. XIII. Influence of Certain Copper Salts Upon Hemoglobin Output, J. Biol. Chem. **79**: 577, 1928.
8. Elvehjem, C. A., Siemers, A., and Mendenhall, D. R.: Effect of Iron and Copper Therapy on Hemoglobin Content of the Blood of Infants, Am. J. Dis. Child. **50**: 28, 1935.
9. Elvehjem, C. A., Duckles, D., and Mendenhall, D. R.: Iron Versus Iron and Copper in the Treatment of Anemia in Infants, Am. J. Dis. Child. **53**: 785, 1937.
10. Usher, S. J., MacDermot, P. N., and Lozinski, E.: Prophylaxis of Simple Anemia in Infancy With Iron and Copper, Am. J. Dis. Child. **49**: 642, 1935.
11. Kropp, G. V., Goodale, R. H., and Cox, C. A.: Observations on the Use of a Copper and Iron Compound in Secondary Anemia, M. Rec. **150**: 67, 1939.
12. Mills, E. S.: The Treatment of Idiopathic (Hypochromic) Anemia With Iron and Copper, Canad. M. A. J. **22**: 175, 1930.
13. Schube, P. G., and Prescott, B. D.: The Effect of Copper and Iron Upon the Secondary Anemia of Therapeutic Malaria in General Paresis, J. LAB. CLIN. MED. **24**: 346, 1939.
14. Bethell, F. H., Goldhamer, S. M., Isaacs, R., and Sturgis, C. C.: The Diagnosis and Treatment of the Iron-Deficiency Anemias, J. A. M. A. **103**: 797, 1934.
15. Duckles, D., Willis, L., and Elvehjem, C. A.: The Treatment of Hypochromic Anemia in College Women, J. Am. Dietet. A. **12**: 537, 1937.
16. Barer, A. P., and Fowler, W. M.: Influence of Copper and a Liver Fraction on Retention of Iron, Arch. Int. Med. **60**: 474, 1937.

IODINE ABSORPTION BY THE SERUM OF EPILEPTIC PATIENTS ON BROMIDE THERAPY*

IRVING C. SHERMAN, M.D., AND ALEX J. ARIEFF, M.D., CHICAGO, ILL.

BECAUSE of the differences of opinion in the literature we were prompted to investigate the iodine absorption values of the serum in a group of epileptic patients on sodium bromide therapy. An effort was made to determine whether the bromide caused any saturation of the unsaturated fatty acids of the serum, and hence be a possible explanation of the common occurrence of skin eruptions on bromide medication. The iodine absorption value was correlated with the level of serum bromide. Cholesterol studies were also done on this group of patients.

During their studies on the nature of serum antitrypsin, Jobling and Petersen[7] showed that the ferment-inhibiting properties of the serum are due to the presence of compounds of the unsaturated fatty acids. They also showed that the iodine absorption value of a serum treated with potassium iodide falls to almost one-fifth of its original value—an effect which is probably due to the liberation of small amounts of free iodine from the potassium iodide which can then saturate the unsaturated carbon bonds. Furthermore, they concluded that the influence of iodine in removing the inhibiting action of serum shows that the inhibiting agents belong to the unsaturated group of fatty acids. From

*From the Department of Nervous and Mental Diseases, Northwestern University Medical School. The Minnie Frances Kleman Memorial Fund.
Received for publication, January 12, 1940.

this was evolved the following explanation for the cause of acne in patients on bromide and iodide therapy. It was suggested that the bromine and iodine radical neutralize the unsaturated fatty acid antitryptic effect. This allows the proteolytic ferment to produce proteotoxins and thus produce the skin eruptions.

Subsequent to this Burr and Burr[1] observed that young rats restricted to fat-free diets do not thrive and develop scaliness of the skin and tail, which could be prevented or alleviated by adding unsaturated fatty acids to the diet. They used linoleic or linolenic acid and termed them "essential fatty acids" because of this ability. In 1933 Hansen[5] investigated the significance of the unsaturated fatty acids in cases of infantile eczema. He showed that in 21 determinations on 10 infants with eczema the amount of iodine absorbed per 100 c.c. of serum ranged between 280 and 487, with an average of 383 mg. per 100 c.c. of serum. In 18 determinations on 16 control infants the amount of iodine absorbed per 100 c.c. of serum ranged between 460 and 711, with an average of 539 mg. per 100 c.c. of serum. The average iodine number of the serum fatty acids in the eczema group was 84, and in the control group was 111. He concluded that the serum fatty acids are more saturated in cases of infantile eczema than in control infants. He[6] also reported that in infants with intractable eczema clinical improvement was shown when oils rich in unsaturated fatty acids were administered internally for various lengths of time. This was associated with a rise to normal of the iodine numbers of the serum lipids. This could not be confirmed by Faber and Roberts,[3] who could find no difference between the incidence of recovery for patients treated this way and those subjected to the usual therapeutic procedures. Taub and Zakon,[8] working on a small series of cases of atopic dermatitis (neurodermatitis), concluded that some patients were actually worse off after taking raw linseed oil internally.

Cornbleet[2] is the only one who confirms Hansen's opinion. He reported uniformly good results when oils rich in unsaturated fatty acids were given to a large series of patients with chronic eczema. This was again denied in 1937 by Ginsberg, Bernstein, and Iob,[4] who could find no characteristic abnormality of the blood lipids in patients treated, or that any specific benefit arises from feeding such patients oils containing highly unsaturated fatty acids.

METHOD

Blood was drawn in the morning on a fasting stomach. The blood was allowed to clot and the serum was removed. One cubic centimeter of serum was extracted with three parts alcohol and one part ether. When cooled, it was made up to a volume of 50 c.c. Ten cubic centimeters of this extract were used for the cholesterol determination according to Bloor's oxidative method. The remainder of the extract was used for the determination of amount of iodine absorbed per 100 c.c. of serum. The blood bromide values were determined in almost all cases on a blood specimen drawn on the same day by the Wuth colorimetric method.

The iodine absorption value of the serum was determined by the micromethod of Rosenmund and Kuhnhenn, as modified by Page, Pasternack, and Burt.[9] Yasuda's[10] technique was used except that 0.01 N thiosulfate was used for titration.

In this work we took the number of milligrams of iodine absorbed per 100 c.c. of serum (after correcting for the cholesterol factor) as being quite indica-

tive of the iodine number of the total unsaturated fatty acids. We believed this tenable because practically the only substances in the serum that will absorb iodine are unsaturated fatty acids and cholesterol. In addition, the alcohol-ether mixture extracts only lipoids. Since the amount of iodine absorbed by the cholesterol was deducted in the formula, it is correct to say that N equals the number of grams of iodine absorbed by the unsaturated fatty acids in 100 c.c. of serum. It has been shown in the work of Hansen and Boyd[6, 11] that the fatty acids are fairly constant in amount in different individuals. In Hansen's infants there was practically no difference in the fatty acid content of the serum regardless of whether they had eczema. Hansen's work clearly indicates that N parallels the iodine number of the fatty acids. We feel justified, therefore, in considering N as being indicative of the degree of unsaturation of the fatty acids in the serum. This made it possible to avoid the long and complicated procedure of actually measuring the quantity of fatty acids.

The figures for N represent the number of milligrams of iodine absorbed by the fatty acids in 100 c.c. of serum, i.e., exclusive of the cholesterol. These are not exactly comparable to those quoted from Hansen because he gave values for the amount of iodine absorbed by both cholesterol and fatty acids.

RESULTS

I. Cholesterol Values.—Cholesterol values were determined in 45 instances on 41 epileptic persons, divided into two groups: (1) those who had not started taking sodium bromide, or whose blood bromide was too low to be estimated; (2) those who had a measurable amount of bromide in the blood.

Group I—11 determinations.

The range of cholesterol was from 171 to 294 mg. per 100 c.c. of serum. The average value was 209. These limits are reasonably close to the normal cholesterol range of 160 to 250 mg. per 100 c.c. of serum.

Group II—34 determinations.

The range of cholesterol was from 132 to 368 mg. per 100 c.c. of serum. The average value was 203. There were only 8 determinations over 250, and 2 of them were on a patient with hypothyroidism. There was no significant alteration in the serum cholesterol values of epileptic persons on sodium bromide therapy.

II. Relationship of Cholesterol Values to Blood Bromide Level.—The patients were grouped into four classes according to the blood bromide level. The cholesterol values in each group were averaged with the following result:

Blood Bromide Range	Average Cholesterol in this Range
75 to 150	240 (6 cases)
151 to 200	230 (15 cases)
201 to 250	240 (8 cases)
251 to 300+	204 (5 cases)

These data indicate that the level of bromide in the blood had no material effect upon the cholesterol value. The group from 251 to 300+ mg. of sodium bromide per 100 c.c. serum had a lower average cholesterol than the others but was well within normal limits and is, therefore, not significant.

III. Relationship of Acne to Blood Bromide and Cholesterol Levels.—A survey of Table I shows distinctly that the severity of the acne is independent

of the blood bromide level. In those patients who have mild acne or seborrheic skin prior to bromide medication it was noticed that the bromide usually caused an exacerbation of the skin lesions. This was independent of the quantity of bromide ingested or of the level to which the blood bromide had been elevated. It was also noted that the age of this susceptible group was roughly in the adolescent and postadolescent period.

TABLE I

Mean iodine value is 508; median iodine value is 527.

CASE NO.	AGE	IODINE ABSORPTION VALUE	SERUM BROMIDE	SERUM CHOLESTEROL	ACNE	REMISSION RATING
1	39	698	–75	203	++	R.X.
2	42	692	150	227	+	F.R.
3	12	661	225	208	++	Re.
4	17	660	0	200	+	--
5	24	660	175	284	±	R.X.
6	52	660	220	344	++	R.X.
7	20	648	115	227	++	F.R.
8	20	597	200	165	+	R.X.
9	29	593	0	200	0	--
10	13	589	0	200	+++	--
11	15	571	210	187	+++	R.X.
12	40	569	–75	294	0	F.R.
13	50	560	185	263	+	F.R.
14	18	554	220	212	+	R.X.
15	27	552	0	212	0	--
16	30	545	250	195	+	F.R.
17	31	545	250	256	+	F.R.
18	33	533	275	208	+	I.R.
19	39	*527*	200	250	+++	Same case as No. 1.
20	27	526	160	250	+	Re.
21	15	*508*	190	297	+	R.X.
22	9	507	165	186	0	I.R.
23	35	503	275	193	+++	F.R.
24	60	495	190	272	0	F.R.
25	19	489	260	227	0	F.R.
26	7	467	250	368	0	I.R.
27	28	464	110	234	0	F.R.
28	17	464	220	188	+	F.R.
29	20	457	160	227	0	R.X.
30	40	438	135	321	+	I.R.
31	50	397	110	208	0	F.R.
32	16	394	0	171	++	—
33	22	371	160	132	++	R.X.
34	52	362	160	216	0	F.R.
35	9	330	300	208	0	F.R.
36	17	314	170	182	++	R.X.
37	17	277	235	150	++	Same as No. 36.
38	21	108	300+	184	+++	R.X.

It is also apparent that there is no relationship between the amount of cholesterol in the blood and the severity of the acne. The patients were classified into four groups, according to the severity of the acne. The average cholesterol value for each group is indicated below:

Acne	Average Cholesterol
0 (No acne)	234 (13 cases)
+ (Mild)	242 (16 cases)
++ (Moderate)	200 (8 cases)
+++ (Severe)	203 (5 cases)

From these data there appears to be some difference between the two extremes of no acne and +++ acne. This difference is of no significance clinically, since both extremes are in the normal range.

IV. Relationship of Iodine Absorption to the Blood Bromide Level.—In 34 patients the iodine absorption value of the fatty acids of the serum was measured on the same specimen of blood as was the blood bromide. These patients had been on sodium bromide for varying periods of time and their blood bromide levels varied from –75 to 300+ mg. of sodium bromide per 100 c.c. of serum. One can see that there is very little relationship between the amount of iodine absorbed by the fatty acids in 100 c.c. of serum and the blood bromide level. A perusal of Table I reveals a range of iodine values from 698 down to 108. The blood bromide values ranged from –75 to 300+. In only very occasional instances was a high iodine value correlated with a low blood bromide value. There were many more instances in which such a correlation did not exist.

V. Relationship of Iodine Absorption Value to the Presence of Acne.—A general survey of Table I shows that there are cases of acne distributed over the whole range of iodine absorption values from 108 to 698. This would, therefore, appear to yield a negative result. That is to say, it is not probable that the bromide taken orally serves to saturate any unsaturated fatty acids and thus inactivate the antitryptic ferments. However, if one derives an average iodine absorption value (508), it is apparent that there are 18 cases of acne above this average and only 8 cases below. This would seem to indicate that there is a general tendency for acne to occur more readily in patients with a high iodine absorption value than in those with a low value. In other words, it appears that the higher the level of unsaturated fatty acids the easier it is for acne to occur. This is exactly opposed to the original theory of Jobling and Petersen.

Table I shows that there are 20 patients in the scale above the average iodine absorption value and 17 below the average. Of the 20 above the average there are 13 under 30 years of age and 8 under 20 years of age, the average age being 27 years. Of the 17 below the average there are 12 under 30 years of age and 9 under 20 years of age, the average age being 26 years. Therefore, it can be stated that the general tendency for greater prevalence of acne in the group with higher iodine absorption values is not determined by the difference in ages of the two groups.

VI. Relationship of Iodine Absorption Values to Remissions of Convulsions.—In this group of patients 31 were followed and classified according to response to treatment.

1. Initial Remission. There were four patients who had a remission over six months of all spells at the onset of treatment. The iodine absorption value ranged from 438 to 533, with an average of 486.

2. Final Remission. Fourteen patients eventually had a remission of all spells lasting six months or more. The range of iodine absorption value was 330 to 692, with an average of 504.

3. Remissions and Exacerbations. Eleven patients showed improvement by remissions of all spells for periods of two to three months alternating with exacerbations of spells. The range of iodine absorption values was from 108 to 660, with an average value of 484.

4. Recalcitrant. In this group of two patients no improvement occurred. The iodine absorption values were 661 and 526, with an average of 593. The group was too small to be of significance.

The values in the first three groups are too close to be of any significance. There is no correlation between iodine absorption value and response to therapy with sodium bromide.

SUMMARY AND CONCLUSIONS

1. The cholesterol values in 41 patients with epilepsy revealed no significant deviation from normal prior to or during therapy with sodium bromide.

2. There was no correlation between the blood bromide level and serum cholesterol in patients on bromide therapy.

3. Acne may occur in patients on bromide therapy regardless of the blood bromide level or serum cholesterol level. Those patients with acneic or seborrheic skin were more prone to an exacerbation of their skin lesions.

4. The iodine absorption value found in our study ranged from 108 to 698, with an average of 508. There was no significant relationship between the blood bromide level and the amount of iodine absorbed by the blood serum.

5. There was no definite correlation between the iodine absorption value and the presence or severity of acne. We did not find that the acne of epileptic patients on bromide therapy was associated with a low level of unsaturation of fatty acids.

6. The response to treatment with sodium bromide was not dependent on the iodine absorption value of the blood.

REFERENCES

1. Burr, G. O., and Burr, M. M.: New Deficiency Disease Produced by Rigid Exclusion of Fat From Diet, J. Biol. Chem. **82**: 345, 1929.
2. Cornbleet, T.: Use of Maise Oil (Unsaturated Fatty Acids) in Treatment of Eczema, Preliminary Report, Arch. Dermat. & Syph. **31**: 224, 1935.
3. Faber, H. K., and Roberts, D. B.: Studies in Infantile Allergic Eczema, Serum Lipids With Special Reference to Saturation of Fatty Acids, J. Pediat. **6**: 490, 1935.
4. Ginsberg, J. E., Bernstein, C., Jr., and Iob, I. V.: Effect of Oils Containing Unsaturated Fatty Acids on Patients With Dermatitis, With Brief Report of Experimental Study in Guinea Pigs, Arch. Dermat. & Syph. **36**: 1033, 1937.
5. Hansen, A. E.: Study of Iodine Number of Serum of Fatty Acids in Infantile Eczema, Proc. Soc. Exper. Biol. & Med. **30**: 1198, 1933.
6. Hansen, A. E.: Serum Lipid Changes and Therapeutic Effects of Various Oils in Infantile Eczema, Proc. Soc. Exper. Biol. & Med. **31**: 160, 1933.
7. Jobling, J. W., and Petersen, W.: The Nature of Serum Antitrypsin, Studies on Ferment Action, J. Exper. Med. **19**: 459, 1914.
8. Taub, S. J., and Zakon, S. J.: Use of Unsaturated Fatty Acids in Treatment of Eczema (Atopic Dermatitis, Neurodermatitis), J. A. M. A. **105**: 1675, 1935.
9. Page, I. H., Pasternack, L., and Burt, M. L.: Über den Transport von Fetten und Lipoiden durch Blut nach Oleingabe, Biochem. Ztschr. **223**: 445, 1930.
10. Yasuda, M.: Determination of Iodine Number of Lipid, J. Biol. Chem. **94**: 401, 1931.
11. Boyd, E. M.: Lipid Analysis of Blood Plasma in Normal Young Women by Microoxidative Methods, J. Biol. Chem. **101**: 323, 1933.

THE STUDY OF ACETYLCHOLINE IN URINE

MIHAJLO MATANOVITCH, M.D., SC.D., PHOENIX, ARIZ.

ACETYLCHOLINE is an organic nitrogenous substance found physiologically in very small amounts in every body cell. It acts as a chemical transmitter of nervous impulses from the motor nerve endings to the muscle end plates.

Elliot was the first to suggest that automatic nerves transmit their impulse at their nerve endings by releasing some specific chemical substance, and Loewi later showed that parasympathetic effects are transmitted by release of acetylcholine. It was found that two chief specific substances were liberated by the nerve fibers: acetylcholine (unstable ester of choline) and an adrenalin-like compound.

Sir Henry Dale called the nerve fibers that liberate the adrenaline-like compound at their terminals "adrenergic" and those that liberate the acetylcholine "cholinergic." Cholinergic fibers are (1) preganglionic fibers of the whole sympathetic system, (2) postganglionic fibers of the parasympathetic system, and (3) motor fibers of the voluntary muscle.

When a preganglionic impulse arrives at the synapse, very small amounts of acetylcholine are liberated to play an important part in transmitting the impulse to the autonomic ganglionic cell and to influence the action of the muscle and gland cells. Feldberg and Dale demonstrated that some postganglionic fibers of the true sympathetic system transmit their effects by means of acetylcholine. Dale thinks that the action of acetylcholine must be on the effector cells and not on the nerve endings. It seems that the transmitting substance is held inactive and is released when the nerve impulse arrives.

Ergehart (1931) showed that this depot of the transmitting substance depends for its maintenance on the integrity of the nerve endings and that it disappears or becomes depleted when the nerve fibers start to degenerate.

Mott and Halliburton were unable to find the choline or its unstable ester in urine with their chemical method. Guggenheim and Loeffler described a biological method by which they were able to extract acetylcholine from urine in the smallest negligible amounts. After numerous experiments they found that a 1:1,000,000,000 solution of synthetic acetylcholine will produce minimal peristaltic movements on the small intestine of a guinea pig, and that the amount of urine necessary to produce same minimal peristalsis should contain 0.000.001 mg. acetylcholine.

Method.—From a twenty-four-hour urine specimen 2 c.c. of urine were collected, evaporated, treated with alcohol and acetylchloride, neutralized, and

Received for publication, January 19, 1940.

finally diluted up to an amount of 100 c.c. This solution was then examined in very small increasing amounts on the small intestine of a guinea pig, and the slightest peristaltic movement was carefully recorded.

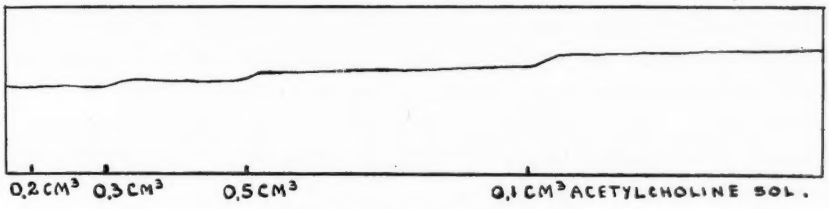


Fig. 1.—Bronchopneumonia.

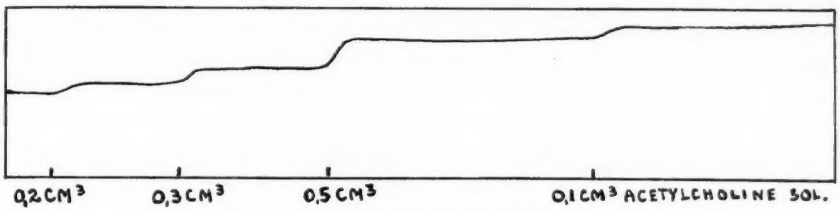


Fig. 2.—Locomotor ataxia.

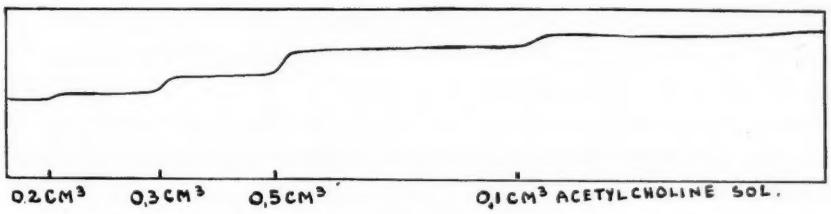


Fig. 3.—Myasthenia gravis.

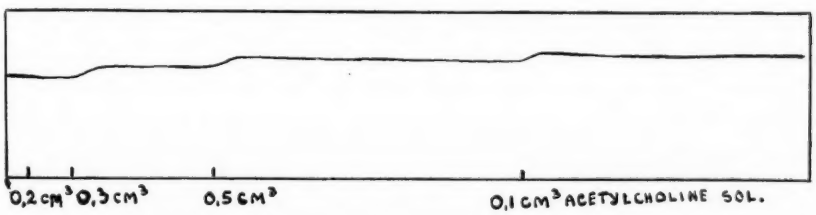


Fig. 4.—Myasthenia gravis (after treatment with prostigmine).

Two cubic centimeters of urine were collected from a twenty-four-hour specimen (800 c.c.) and treated by the above-mentioned method. Choline was transformed into acetylcholine, neutralized, diluted to 100 c.c., and the resulting solution examined on the intestine of the guinea pig (Fig. 1). Two-tenths cubic centimeter of the solution did not record any peristaltic movement; 0.3 c.c. of the solution recorded minimal peristalsis; 0.5 c.c. of the solution recorded strong peristalsis; and 0.1 c.c. of acetylcholine solution that contains 0.000.001 mg. of acetylcholine recorded the same peristaltic movements as 0.3 c.c. of the examining solution, which contains the same amount of 0.000.001 mg. of acetylcholine. The whole amount (100 c.c.) of the examining solution contains $\frac{0.0001}{0.3}$

or 0.00033 mg. of acetylcholine. As 100 c.c. of the examining solution was prepared from 2 c.c. of a twenty-four-hour urine specimen (800 c.c.), the amount of acetylcholine in the whole daily specimen will be: $0.0003 \times \frac{800}{2} = 0.132$ mg.

Making a study with the foregoing method, I extracted choline in the form of acetylcholine from the urine of 120 patients. In patients suffering from nephritis, arteriosclerosis, and vitium cordis, acetylcholine was found in very small amounts in urine. In five patients with myasthenia gravis treated with prostigmine, acetylcholine was eliminated in urine in very negligible amounts (0.016 to 0.100 mg.).

It was very interesting to find that choline and acetylcholine were eliminated in larger amounts in the urine of patients suffering from parasyphilitic diseases of the brain and spinal cord. In six persons with locomotor ataxia and one with general paresis with positive Wassermann test, acetylcholine was found in urine in very large amounts. In three patients with myasthenia gravis acetylcholine was eliminated in urine in surprisingly large amounts of 0.350 mg., 0.450 mg., and 0.500 mg. (in a twenty-four-hour urine specimen).

CONCLUSION

From my experiments it is evident that choline and its unstable ester "acetylcholine" stored in the nerve fibers become depleted and are eliminated in the urine in unusually large amounts in patients suffering from parasyphilitic diseases of the brain and spinal cord.

It seems that in myasthenia gravis accelerated destructive action of choline esterase is inhibited by the administration of prostigmine and the elimination of the substance in the urine is greatly decreased.

REFERENCES

1. Dale, Sir Henry: Chemical Transmission of the Effects of Nerve Impulses, Brit. M. J. p. 835, May 12, 1934.
2. Dale, Sir Henry: Pharmacology and Nerve-Endings, Proc. Roy. Soc. Med. **28**: 319, 1935.
3. Loewi, Otto: Ueber humorale Uebertraegbarkeit und Herznervenwirkung, Pflüger's Arch. **199**: 239, 1921.
4. Loewi, Otto: Chemical Transmission of Nervous Impulse, Proc. Roy. Soc., London, s.B. **118**: 299, 1935.
5. Dale, H., Feldberg, W., and Vogt, M.: Release of Acetylcholine at Voluntary Motor Nerve Endings, J. Physiol. **86**: 353, 1936.
6. von Guggenheim, O., and Loeffler, W.: Ueber das Vorkomen und das Schicksal des Cholins im Tierkoerper, Biochem. Ztschr. **74**: 208, 1916.
7. Matanovitch, M.: Cholingehalt des Harnes unter pathologischen Bedingungen, Med. Clinic Basel, Dec. 28, 1918.
8. Pritchard, E. A. Blake: Prostigmin in the Treatment of Myasthenia Gravis, Lancet **1**: 434, 1935.

910 Professional Bldg.

CHOLINESTERASE DETERMINATIONS*

ROLAND C. HAWES, B.S., AND GORDON A. ALLES, PH.D., LOS ANGELES, CALIF.

NUMEROUS methods have been proposed and used for the estimation of the cholinesterase content of blood or tissues. Their variety has resulted from most investigators in the field making an arbitrary choice with regard to one or more of the several variables that affect cholinesterase activity. The methods fall into two classes, depending upon whether bio-assay is used to measure residual acetylcholine after enzyme action has occurred, or whether the acetic acid produced is estimated by a suitable chemical method.

The two classes of methods differ in some important respects. The bio-assay methods are usually carried out at low substrate concentrations, the reaction periods are short (often only a few seconds), the methods are laborious, and the precision of estimation of extent of hydrolysis per unit time is low. However, bio-assay methods may provide the nearest laboratory approach to the study of enzyme kinetics under physiologic conditions. The chemical methods are limited by their relative insensitivity to use of high substrate concentrations, but are capable of much greater precision. The flexibility and simplicity of chemical methods encouraged us to investigate the conditions under which they provide a satisfactory measure of the cholinesterase activity of blood.

A study of the cholinesterase activity of human blood preparations was reported[1] in which we were able to show that there are at least two enzymes possessing such activity in blood. One appears in the serum and the other in the cells, and the latter may be studied with solutions or suspensions of cells after centrifuging then washing. The effects of several variables affecting cholinesterase estimation techniques were also investigated. The present paper applies these studies to the problem of practical use of this type of assay and indicates the clinical situations in which cholinesterase determinations have given interesting results.

Over a wide range of conditions whole blood activity was found to be the sum of the separate activities of the cell and serum enzymes.[1] The cell enzyme activity markedly predominates over that of the serum as the substrate concentration is diminished to about that at which the enzymes must act in the body. The cell enzyme is inhibited by excess acetylcholine substrate, promoted by sodium ion in physiologic salt solution, and little affected by pH changes of the solution. The serum enzyme activity increases approximately with the substrate concentration up to 0.01 millimolar, and no inhibition is noted at higher concentrations.[2-5] The serum enzyme has a pH optimum[6] at about 8.0 and is inhibited only slightly by 0.85 per cent sodium chloride concentration.

*From the Laboratories of George Piness, M.D., and Hyman Miller, M.D., Los Angeles.
Received for publication, January 29, 1940.

The influence of substrate concentration on rate and the inhibition of both enzymes by choline ion make it desirable to perform titrations at high substrate concentrations. The standard conditions that have been selected for the present titration method allow it to be readily applied to serum, cell, or whole blood samples with a high degree of precision.

METHOD

Blood for whole blood titrations is drawn from finger or ear prick into calibrated 0.2 ml. pipettes and immediately rinsed into 4.8 ml. of water containing 1:100,000 phenylmercuric acetate as a preservative. It may be stored on ice for a week or more before testing. For separated serum and cell titrations the technique already described[1] is satisfactory, or blood may be drawn as above, rinsed into 4.8 ml. of saline, centrifuged, and the cells washed once with saline, then laked in water to make 5 ml. The difference in sodium chloride content of the two fractions has only a slight effect on the serum activity (decrease of about 10 per cent). Possibly a better method would be to do all titrations at a final sodium chloride content of 0.85 per cent, but unfortunately the important influence of salts on the enzymes was not recognized before we had obtained our data on normal values of whole blood, serum, and cells.[1, 7] Since the values obtained can in any case have only comparative significance (see Discussion), it has not seemed worth while to repeat our work.

The titration is performed with the aid of a commercial glass electrode pH meter (Beckman model G). A test tube of 40 ml. capacity placed in a bath at 37° C., or jacketed and warmed with circulating water from a thermostat, serves as the reaction vessel. The contents are stirred by hand with a large glass bulb blown on the end of a piece of narrow tubing. The stirrer occupies 10 ml. of the volume. The electrodes are immersed in the reaction mixture to a depth of about one inch and kept there several minutes before taking readings in order to bring their contents to temperature equilibrium. It is necessary to use an excess of solid potassium chloride in the calomel half cell and to rerinse the liquid junction after temperature equilibrium is reached to keep the bridge solution saturated throughout. It is convenient to connect the electrodes to the pH meter by means of the special cable which is available. Standardized 0.02 normal sodium hydroxide is added as required from a 5 ml. burette with a capillary tip immersed in the reaction solution. A stock 0.02 molar acetylcholine solution is used which contains 5.46 Gm. of acetylcholine iodide in a liter of distilled water and is adjusted to pH 4.0 by the addition of 0.1 normal hydrochloric acid. This stock solution is kept in the dark in a tightly stoppered pyrex flask and is stable for three months or more.

The enzyme solution (5 ml.) and the acetylcholine iodide stock solution (20 ml.), separately warmed, are mixed in the reaction vessel and adjusted to pH 7.40. The burette is read and the pH is kept within 7.30 and 7.50 by small additions of standardized alkali. The time required for each 0.1 or 0.2 ml. increment of alkali is recorded to the nearest second. These should be constant within the reading errors. After about ten minutes the precision of estimation should be better than ±2 per cent.

The amount of alkali used per twenty minutes[8] is calculated, and a blank of 0.085 ml. is subtracted from this value to allow for the alkaline hydrolysis. The result is corrected for any pipette error to obtain the index of activity or "rate."

NORMAL VALUES

These data have been presented previously[1, 7] and are collected here for convenient reference. In Tables I and II are shown the results of a number of individual determinations made on normal persons, *s* being the estimate of the standard deviation of their distribution.[18] The data of Table I are grouped to permit an analysis of age or sex differences, and it is to be noted that no significant age or sex differences were found. In Table II repeated determinations on four normal persons are tabulated, and the data show that change of enzyme in a single person with time is confined to a range of variation only one-third of the variation from person to person.

In Table III is shown the distribution of enzyme of whole blood into serum and cell fractions and the ratio of serum to whole blood enzyme activities.

DISCUSSION

Perhaps more important than the method itself are two questions regarding the results which may be obtained: first, when may they be expected to be profitable? and second, what significance can be attributed to them? As an attempt to answer the first question, we have appended a summary of the literature on blood cholinesterase determinations of clinical and physiologic interest in the light of present knowledge of these enzymes. For the second, there are certain considerations that may assist the clinician in deciding what his results mean.

It is clear that present technique and knowledge do not permit physiologic interpretation of the results of blood or blood fraction assay. Other tissues are known to possess active enzymes, which have not been clearly characterized, and acetylcholine is readily diffusible. The only study of acetylcholine-cholinesterase physiology with which we are acquainted that seems reasonably free of critical objection is that of Glick,[9] who worked, over a considerable range of conditions, with tissue from the superior cervical ganglion of the cat and confined his conclusions to the same organ. Significance of results must, therefore, be based on a comparison with normal values, or with a normal level established for the person studied in case it is desired to evaluate the results of therapy.

For the purpose of such comparison it has seemed to us that whole blood titrations have practically the same utility as blood fraction titrations. Under our conditions each enzyme contributes nearly the same share of the whole blood value. The probability of encountering compensatory changes in both enzymes seems negligible in view of the considerable differences in their behavior and of their demonstrated independence.

A second important consideration is occasioned by the relatively wide range of activities we, as well as others, found in ostensibly normal persons. The extremes in rates we found in about 100 assays of normal whole bloods were 0.500 to 1.068. If the rates are normally distributed about a mean of

TABLE I

TITRATIONS OF NORMAL PERSONS

PERSON	MALES AGE	RATE	PERSON	FEMALES AGE	RATE
E. V.	14	0.501	V. K.	26	0.792
			M. K.	26	0.748
			M. A.	32	0.747
B. W.	34	0.947	C. F.	24	0.716
C. B.	29	0.846	L. F.	23	0.735
C. C.	24	0.670	P. G.	22	0.542
R. W.	30	0.821	F. B.	25	0.633
D. F.	25	0.700	R. L.	24	0.805
	Mean of group	0.797		Mean of group	0.714
G. A.	36	0.780			
H. M.	40	0.902	E. J.	38	0.714
G. P.	46	0.876	G. W.	35	0.895
F. K.	36	0.633	C. S.	45	0.986
P. K.	35	0.724			
	Mean of group	0.783			
G. M.	66	0.554	S. R.	58	0.803
S. M.	52	0.934	Y. R.	66	0.919
	Mean of males	0.761		Mean of females	0.772
	s	0.144		s	0.118
	Mean of normals	0.766			
	s	0.129			

TABLE II

TEMPORAL VARIATION OF ENZYME

BLEEDING DATE	B. W. (MALE) AGE 34	E. J. (FEMALE) AGE 38	V. K. (FEMALE) AGE 26	M. K. (FEMALE) AGE 26
9/21/36	0.865			
10/ 5/36	0.967	0.736	0.785	0.703
10/ 6/36	0.918	0.714	0.736	0.735
10/ 7/36	0.971	0.687	0.838	0.763
10/ 9/36	0.910	0.698	0.825	0.724
10/10/36	1.009		0.788	0.737
10/13/36	0.968	0.810	0.793	0.805
10/20/36	0.915	0.705	0.765	0.710
10/27/36	0.981	0.702	0.822	0.814
11/ 3/36	0.911	0.736	0.754	0.720
11/13/36	0.931		0.754	0.746
11/25/36	0.960	0.704	0.803	0.750
12/14/36			0.834	0.739
12/17/36	0.965	0.652		
1/30/37	0.994	0.707	0.805	0.777
Means	0.9469	0.7137	0.7923	0.7479
s values	0.034	0.038	0.048	0.044

TABLE III

DISTRIBUTION OF ENZYME IN BLOOD

SUBJECT	FROM 0.2 ML. BLOOD		SERUM CELLS	WHOLE BLOOD 0.2 ML.	RATIO SERUM/BLOOD
	SERUM	CELLS			
Males					
G. A.	0.50	0.30	0.80	0.80	0.62
H. M.	0.55	0.31	0.86	0.90	0.61
C. B.	0.47	0.35	0.82	0.84	0.56
G. P.	0.49	0.36	0.85	0.87	0.56
Females					
M. K.	0.43	0.34	0.77	0.74	0.58
V. K.	0.42	0.35	0.77	0.83	0.51
M. B.	0.39	0.28	0.67	0.65	0.60
Averages	0.46	0.33	0.79	0.80	0.58

0.76, with a standard deviation of 0.13, these limits would be expected to be equaled or exceeded about three times in 100 trials. Corresponding range limits for one time in 1,000, 100, and 20 are 0.33 to 1.19, 0.42 to 1.10, and 0.50 to 1.02, respectively.

These figures enable one to make an estimate of the possible significance of a single individual determination. For example, in assays on ten patients hospitalized for various conditions (unpublished data obtained through the cooperation of Dr. S. R. Baker of the Cedars of Lebanon Hospital, Los Angeles), the three lowest and the two highest values were 0.43 (coronary artery disease), 0.46 (spinal tuberculosis), 0.50 (subacute bacterial endocarditis), 1.04 (rheumatic heart disease), and 1.06 (hyperthyroidism). None of these can be considered individually significant or of diagnostic value, but they suggest that studies of these conditions might be of interest, and, indeed, in tuberculosis and in hyperthyroidism significant changes of activity have been demonstrated (see below) by comparing the average from a number of persons so afflicted with an average for normals. The range of variation of average values to be expected is much smaller than that for individual determinations and may be made as small as is desired by including a sufficient number of individuals in the groups. So far as we are aware, there is today no condition known for which the assay of blood cholinesterases can be expected to give individual results of consistent diagnostic value. It appears, however, that the possible prognostic value of individual determinations has not been explored.

CHOLINESTERASE IN DISEASE AND OTHER CONDITIONS

Ginsberg, Kohn, and Necheles[10] reviewed much of the literature on blood cholinesterase determinations and came to the conclusion that a definite change had been demonstrated only in tuberculosis. However, it appears certain that the function of the (cholinergic) motor nerves is disturbed in myasthenia gravis. Walker[11] found that administration of cholinesterase inhibitors produces prompt but temporary relief of the symptoms of the disease. McGeorge[12] observed that the period of symptomatic improvement after giving prostigmine coincides with the period of decreased activity of the serum enzyme, as determined by the Stedman gasometric method. Fraser, McGeorge, and Murphy[13] found that large doses of choline esters, including acetylcholine itself (given as the chloride), produced a delayed, prolonged period of relief, which they explained by postulating the utilization of some of the acetylcholine in formation of the acetylcholine precursor in the nervous tissue, since choline ion, with or without acetate, produced no such effect. Ingvarsson[14] showed that a single large dose of acetylcholine iodide does not alter the activity of cholinesterase in whole blood, as determined by a modification of the Galehr and Plattner[15] bio-assay method. Hall and Ettinger[16] found that in dogs repeated large daily doses produced no change in serum enzyme.

With regard to the cholinesterases in the blood of myasthenic persons, there is a considerable divergence of results. Stedman and Russell[17] demonstrated conclusively that, when estimated by Stedman's gasometric method, there is a significant decrease in the serum cholinesterase, while the corpuscle cholinesterase is normal in amount, as compared with normal individuals.

Fisher's "t" test[18] applied to the difference between the arithmetic averages of the values of serum cholinesterase reported for 12 myasthenic and nine normal individuals, shows that a difference of this magnitude would have been obtained by accident only about one time in 50 if myasthenic persons were really no different in this respect from normal individuals (our own calculation). Two myasthenic persons, followed for a considerable period of time, showed a marked drop in blood cell cholinesterase values after spontaneous remissions of symptoms.

McGeorge's[12] observation that in three myasthenic persons the serum enzyme, measured by the Stedman titration method, was within the normal range, cannot be taken as a contradiction of the results of Stedman and Russell, since the values reported by the latter for normal and myasthenic persons overlapped. If Stedman and Russell's figures are representative, the number of individuals they measured is about as few as could be relied upon to show a significant difference. On the other hand, Hicks and MacKay[19] found very large increases in serum cholinesterase in three myasthenic persons as compared with 20 normal persons. However, they failed to present data to show that the reliability and reproducibility of the bio-assay method which they used is adequate to demonstrate with certainty differences of the magnitude they found, especially since practically the whole of the discrimination depended upon differences in the rates of hydrolysis within the first five seconds of mixing the reagents. Furthermore, they used prostigmine to stop the reaction after it had proceeded for the desired time intervals, without presenting experimental justification for their assumption that the prostigmine acts practically instantaneously. Easson and Stedman[20] have shown that the reaction between this same inhibitor and an enzyme derived from horse serum by the Stedman and Stedman[21] purification method requires several hours to reach equilibrium. Matthes[22] also indicated that appreciable time was required to reach equilibrium in the reaction of eserine with whole blood.

Hicks and MacKay accounted for the difference between their results and those of Stedman and Russell by pointing out that their measurements were made under different conditions from those of Stedman and Russell. However, our experiments on the behavior of human serum enzyme with changes of pH and of substrate concentration show that this explanation is probably not valid, and that, should the observations of Hicks and MacKay be capable of critical verification, it will be a strong indication that the cholinesterase of myopathic sera differs qualitatively from that of normal serum.

Corkill and Ennor[23] made measurements of the total activity of whole blood of two myasthenic persons as compared with a group of normal persons, using a bio-assay method to estimate the residual acetylcholine ion concentration after the enzyme had acted for twenty seconds and had been stopped by the addition of trichloracetic acid (Galehr and Plattner,[15] and Matthes[22]). The myasthenic bloods fell well within the normal range of activity. This corroborates the findings of Stedman and Russell[17] that the blood cell enzyme is within the normal range, since the measurements were made at a concentration of acetylcholine ion (approximately 0.0005 molar) for which we have found the serum enzyme contributes but little to the activity of whole blood.

Russell and Stedman[24] observed that the serum enzyme was low, in one patient with myotonia congenita, while the blood cell enzyme was normal in amount. Hicks and MacKay[19] found five myotonia congenita patients to have a low serum enzyme. The figures available so far cannot be considered more than suggestive evidence of a changed cholinesterase situation in this condition.

In hyperthyroidism Antopol, Tuchman, and Schifrin[25] have shown that the cholinesterase of serum is markedly increased. A group of treated patients showed lower values. The same authors[26] have also published data showing serum values to be lowered in jaundice and biliary disease, and promise data showing decreased enzyme activity in several other conditions. They attribute the lowered values in jaundice to inhibition by bile salts.[27] However, their suggestion that the altered serum esterase may be related to nervous function ("sympathicotonicity or vagotonicity") must be considered at present as purely speculative, in view of the fact that no correlation between serum, blood cell, and tissue enzyme activity has been demonstrated. The same considerations apply to the observations of Tod and Jones[28, 29] that the serum enzyme is significantly altered in certain mental states.

Investigators have been unable to find changes in the cholinesterases in a number of other disease conditions and physiologic states. Altenburger[30] found normal values in whole blood or spinal fluid of patients with epilepsy and with Parkinsonism, using a bio-assay method. We also found no change in titration values under our standard conditions of whole blood in a group of eight Parkinsonism patients under the care of Dr. E. Ziskind at the Los Angeles General Hospital (unpublished data). Hall and Lucas[31] could find no correlation between serum cholinesterase (measured by a modification of the Stedman titration procedure) and age, sex, weight, exercise, fatigue, diet, blood pressure, resting heart rate, hemoglobin, and blood cell counts, or in women during menstruation or pregnancy in 40 normal and 162 hospital patients. Among the hospital patients they could find no correlation with the disease represented. Milhorat[32] made a series of measurements of serum enzyme and arrived at similar conclusions. Obviously, these investigators may have overlooked significant alterations in total blood activity, because they did not include blood cell activity in their measurements.

We have been unable to find any shift in whole blood enzymes in allergic individuals or in asthmatic persons during attacks.[7]

We studied the effect of exercise upon cholinesterase content of the blood. It is known that both the motor nerve mechanism and the reflex vagus inhibition of the heart resulting from blood pressure rise act by acetylcholine mediation, and it seemed not unlikely that the increase of metabolism and blood pressure consequent to violent exercise might be reflected in changes of blood cholinesterases. Total blood cholinesterase activity measurements were made with our standard method before exercise, fifteen minutes after exercise (the time when blood lactic acid is maximal, according to Long and Lupton[33]), and several hours later. The exercise was brief and sufficiently vigorous to more than double the heart rates in two normal adult males. No shifts beyond the limit of error of the method were found (unpublished).

The experiments of Manning, Lang, and Hall[34] on the inhibition of serum cholinesterase by eserine are interpreted by them as indicating that the ac-

cepted theory which attributes the physiologic action of eserine wholly to its inhibitory action on cholinesterase may be in error. This cannot be taken to prove their point, since Ammon and Voss[35] found that the cholinesterases of whole blood do not parallel serum enzyme in their inhibition by eserine. Even if experiments with whole blood enzymes were found to give similar results, there would be no certainty that the enzyme of the tissues was likewise affected.

Dikshit and Mahal[36] demonstrated that the enzymes of whole blood are markedly affected by infection with *B. pestis* or vaccination with plague vaccine, which they interpret as a response to the toxemia and suggest that it may account for the muscular weakness of toxemias. They measured the enzymes both by a bio-assay method at low acetylcholine ion concentrations and by the Stedman gasometric procedure.[37] They found the agreement between the two methods to be improved by decreasing the substrate concentration in the latter method. From our work it would appear that this is a reflection of the increasingly predominant activity of the blood cell enzyme at lower substrate concentrations. The data suggest that the changes they observed were greater for the cell than the serum enzymes.

Other reports, whose expressed or implied conclusions depend on a parallelism in behavior between serum and blood cell or tissue enzymes, are the work of Glick and Antopol[38] on inhibition of cholinesterase activity by vitamin B_1, and of Minot[39] on the influence of toxic doses of guanidine on dog serum cholinesterase. Govaerts, Cambier, and van Dooren[40] concluded that varying degrees of individual sensitivity to acetylcholine were not dependent on differences in rate of destruction of the drug, because such differences could not be found in the plasmas. Ingvarsson's[14] study with whole blood makes this conclusion appear more probable, but it should be noted that the conditions selected for esterase assay were far removed from those under which the enzyme acts in vivo, and may have prevented the detection of significant differences, even if one assumes that destruction in the blood stream is the only factor other than inequality of response that may affect sensitivity.

REFERENCES

1. Alles, G. A., and Hawes, R. C.: Cholinesterases in the Blood of Man, J. Biol. Chem. **133**: 375, 1940.
2. Easson, L. H., and Stedman, E.: Absolute Activity of Cholinesterase, Proc. Roy. Soc., London, s.B. **121**: 142, 1936.
3. Clark, A. J., Raventos, J., Stedman, E., and Stedman, E.: Kinetics of Cholinesterase, Quart. J. Exper. Physiol. **28**: 77, 1938.
4. Roepke, M. H.: A Study of Cholinesterase, J. Pharmacol. & Exper. Therap. **59**: 264, 1937.
5. Ziff, M., Jahn, F. P., and Renshaw, R. R.: The Acetylcholine-Cholinesterase System, J. Am. Chem. Soc. **60**: 178, 1938.
6. Glick, D.: Studies on Enzymatic Histochemistry. XXV. A Micro Method for Determination of Choline Esterase, and the Activity-pH Relationship of This Enzyme, J. Gen. Physiol. **21**: 289, 1938.
7. Hawes, R. C., and Alles, G. A.: Cholinesterase in the Blood of Allergic Patients, J. Allergy **12**: 1, 1940.
8. Stedman, E., Stedman, E., and Easson, L. H.: An Enzyme Present in the Blood Serum of the Horse, Biochem. J. **26**: 2056, 1932.
9. Glick, D.: Choline Esterase and the Theory of Chemical Mediation of Nerve Impulses, J. Gen. Physiol. **21**: 431, 1938.
10. Ginsberg, R., Kohn, R., and Necheles, H.: Studies on the Blood Esterase, Am. J. Digest. Dis. & Nutrition **4**: 154, 1937.
11. Walker, M. B.: Case Showing Effect of Prostigmine in Myasthenia Gravis, Proc. Roy. Soc. Med. **28**: 759, 1935.

12. McGeorge, M.: Cholinesterase Activity in Disease, With Special Reference to Myasthenia Gravis, Lancet **1**: 69, 1937.
13. Fraser, F. R., McGeorge, M., and Murphy, G. E.: The Action of Choline Esters in Myasthenia Gravis, Clin. Sc. **3**: 77, 1937.
14. Ingvarsson, G.: Studies on Acetylcholine Sensitivity and the Velocity of Acetylcholine Splitting by the Human Blood, Biochem. Ztschr. **281**: 370, 1935.
15. Galehr, O., and Plattner, F.: Fate of Acetylcholine in the Blood, Arch. f. d. ges. Physiol. **218**: 488, 1928.
16. Hall, G. E., and Ettinger, G. H.: The Effect of Regular Injections of Acetylcholine Upon the Choline-Esterase Activity of Serum, J. Pharmacol. & Exper. Therap. **59**: 29, 1937.
17. Stedman, E., and Russell, W. R.: The Cholinesterase Content of Blood in Myasthenia Gravis, Biochem. J. **31**: 1987, 1937.
18. Fisher, R. A.: Statistical Methods for Research Workers, ed. 4, London, 1932, Oliver and Boyd, p. 114.
19. Hicks, C. S., and MacKay, M. E.: Cholinesterase Activity of Serum in Two Cases of Myasthenia Gravis, Australian J. Exper. Biol. & M. Sc. **14**: 275, 1936; The Cholinesterase Content of Blood Serums From Normal and Myopathic Subjects, Ibid. **16**: 39, 1938.
20. Easson, L. H., and Stedman, E.: Absolute Activity of Cholinesterase, Proc. Roy. Soc., London, s.B. **121**: 142, 1936.
21. Stedman, E., and Stedman, E.: The Purification of Choline Esterase, Biochem. J. **29**: 2563, 1935.
22. Matthes, M.: The Action of Blood on Acetylcholine, J. Physiol. **70**: 338, 1930.
23. Corkill, A. B., and Ennor, A. H.: Choline Esterase in Myasthenia Gravis, M. J. Australia **2**: 1121, 1937.
24. Russell, W. R., and Stedman, E.: Observations on Myotonia, Lancet **2**: 742, 1936.
25. Antopol, W., Tuchman, L., and Schifrin, A.: Choline-Esterase Activity of Human Serums With Special Reference to Hyperthyroidism, Proc. Soc. Exper. Biol. & Med. **36**: 46, 1937.
26. Antopol, W., Schifrin, A., and Tuchman, L.: Decreased Cholinesterase Activity of Serum in Jaundice and in Biliary Disease, Proc. Soc. Exper. Biol. & Med. **38**: 363, 1938.
27. Sobotka, H., and Antopol, W.: Inhibitors of Choline Esterase, Enzymologia **4**: 189, 1937.
28. Tod, H., and Jones, M. S.: A Study of the Cholinesterase Activity in Nervous and Mental Disorders, Quart. J. Med. **6**: 1, 1937.
29. Jones, M. S., and Tod, H.: The Effect of Altering Conditions of the Autonomic Nervous System on the Cholinesterase Level in Human Blood Serum, J. Ment. Sc. **83**: 202, 1937.
30. Altenburger, H.: Acetylcholine Breakdown in the Blood and Cerebro-Spinal Fluid, Klin. Wchnschr. **16**: 398, 1937.
31. Hall, G. E., and Lucas, C. C.: Cholinesterase Activity of Normal and Pathological Serums, J. Pharmacol. & Exper. Therap. **59**: 34, 1937.
32. Milhorat, A. T.: The Choline-Esterase Activity of the Blood Serum in Disease, J. Clin. Investigation **17**: 649, 1938.
33. Long, C., and Lupton, H.: Fate of Lactic Acid After Exercise, J. Physiol. **67**: 57, 1923.
34. Manning, G. W., Lang, J., and Hall, G. E.: The In Vivo Effects of Eserine on the Choline-Esterase System, J. Pharmacol. & Exper. Therap. **61**: 350, 1937.
35. Ammon, R., and Voss, G.: The Acetylcholine-Destroying Action of Blood, Arch. f. d. ges. Physiol. **235**: 393, 1935.
36. Dikshit, B. B., and Mahal, H. S.: Choline Esterase in Toxemia, Quart. J. Exper. Physiol. **27**: 41, 1937.
37. Stedman, E., and Stedman, E.: The Relative Choline-Esterase Activities of the Serum and Corpuscles From the Blood of Certain Species, Biochem. J. **29**: 2107, 1935.
38. Glick, D., and Antopol, W.: Inhibition of Cholinesterase by Thiamine (Vitamine B_1), J. Pharmacol. & Exper. Therap. **65**: 389, 1939.
39. Minot, A. S.: A Comparison of the Actions of Prostigmine and Guanidine on the Activity of Choline Esterase in the Blood Serum, J. Pharmacol. & Exper. Therap. **66**: 453, 1939.
40. Govaerts, P., Cambier, P., and van Dooren, F.: The Rate of Destruction of Acetylcholine in the Blood of Individuals Sensitive or Resistant to That Substance, Compt. rend. Soc. de biol. **108**: 1178, 1931.

LABORATORY METHODS

GENERAL

IMPROVED INTUBATION TUBES FOR INSUFFLATION ANESTHESIA IN DOGS*

V. Brown Scott, Ph.D., M.D., and Hans Rudolph
Bloomington, Ind.

SINCE pneumothorax results in bilateral lung collapse in the dog, intrathoracic surgery must be performed under some form of intratracheal insufflation. The simplest method consists of periodic inflation of the lungs by blowing air ether intratracheally. A close-fitting intubation tube[1] or the introduction of a tracheal cannula is, however, required.

Hamilton, Woodbury, and Vogt[2] (1939) advocate, for intrathoracic operations, an interrupted blast of air via a small tracheal catheter. This catheter carries a balloon, inflated through an opening in the catheter above its terminal one, which raises the pressure in the trachea during each blast. This method is said to be an improvement over other methods.

Livingstone and Hrdina[3] (1930) developed a "modified Meltzer apparatus" which has been used successfully in their laboratory for many years. This *simple* apparatus delivers ether air at low pressures (compressed air passes through an ether "bottle") to the bifurcation of the dog's trachea via an urethral catheter (No. 16 French). Desired inflation of the lungs is accomplished by impeding the exhaust of gases through the trachea. This is accomplished by a modified Kunde mask into which the dog's muzzle is introduced through a perforation in a tight-fitting rubber dam. The other end of the mask (metal can) has two openings; one opening allows the passage of the insufflating catheter, the other is connected with a tube which leads the exhaust gases into an 8 liter water bottle. The catheter passes intrabuccally through a metal airway.

We have had occasion to use the above apparatus repeatedly in intrathoracic operations. The method has proved essentially satisfactory since one can easily maintain *apnea vagi* by moderate distention of the lungs. No untoward results of this anesthesia have been encountered, and the motionless thorax is a distinct advantage in these operations. We have found, however, disadvantages in the use of the Kunde mask, flexible catheter, and airway used by Livingstone and Hrdina.

Ether anesthesia for most operations in the dog is, we believe, given most satisfactorily by a tight-fitting metal intubation tube[1] which is introduced into the trachea through the larynx. A set of six twelve-inch tubes, ranging from $\frac{3}{4}$ to $\frac{7}{16}$ inches (outside diameter), will be satisfactory for dogs weighing less

*From the Department of Physiology, Indiana University School of Medicine, Bloomington.
Received for publication, February 26, 1940.

than 20 kg. Such tubes can be equipped with a beveled tip (to facilitate intubation) and nickel or chrome plated. Such a tube connected with an ordinary ether "bottle" by a short rubber tube eliminates the bulky Kunde mask and has proved entirely satisfactory. Atropine, preoperatively, is unnecessary.

Satisfactory anesthesia with the metal tubes led us to devise intubation tubes which are used for intratracheal insufflation anesthesia and replace the Kunde mask, metal airway, and long catheter used by Livingstone and Hrdina. This not only eliminates bulky apparatus but makes intubation much simpler.

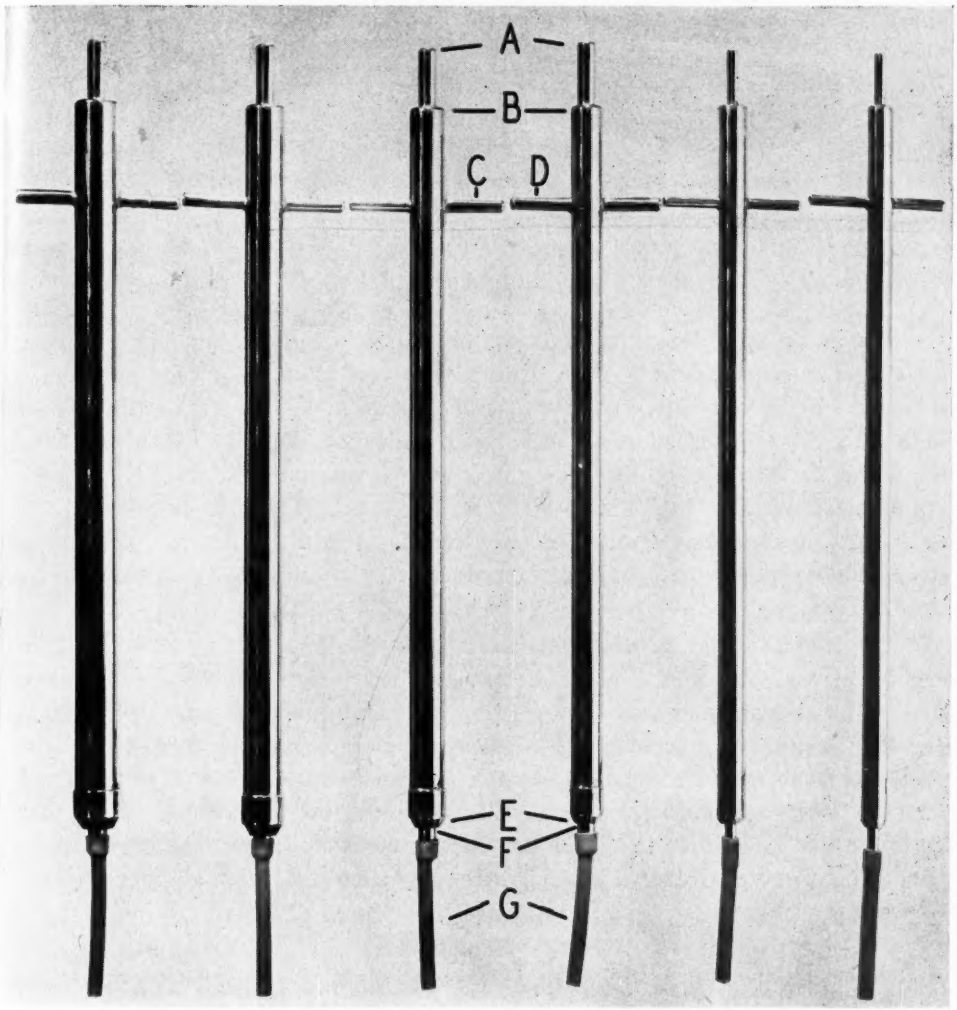


Fig. 1.

APPARATUS

The intubation tubes (Fig. 1) consist of six nickel-plated brass tubes, 12 inches long (standard seamless brass tubing of outside diameters: 3/4, 11/16, 5/8, 9/16, 1/2, 7/16 inches). A smaller tube (3/16 to 5/16 inches, outside diameter), 13 1/2 inches long (*A-F*), is fixed inside the larger tube with *A* (upper end) protruding

1 inch while *F* (lower end) extends ¾ inch below the beveled tip *E*. The outer tube is closed completely about the smaller tube at *B*. Side tube *C* is closed but *D* communicates only with the space between the long inner and outer tubes. The lower end of the outer tube is fitted with a beveled tip *E* (see Fig. 2) which leaves an opening between the tubes. A 3 inch rubber tube *G* is attached to the inside tube *F*.

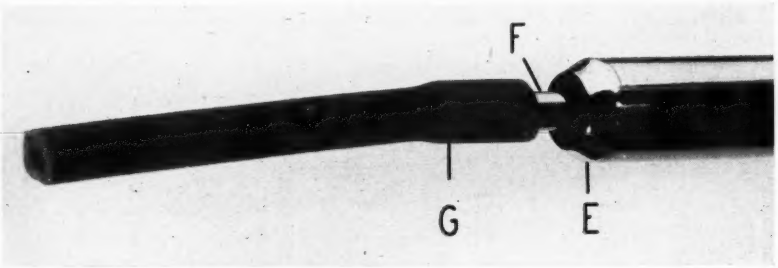


Fig. 2.

METHOD

One hour after administration of morphine sulfate (1 mg. per kilogram) the dog is anesthetized with ether. Under *deep* anesthesia the lower end of the intubation tube is introduced through the larynx on *inspiration* (the tube selected should fit the trachea snugly). The intubation tube is passed caudad so that the tip of rubber tube *G* lies just above the bifurcation of the trachea. Tube *A* is connected with the compressed air-ether source, and *D* is connected with the exhaust tube leading to the water bottle. The dog's jaws are bound tightly about the intubation tube, the bandage being carried around side tubes *C* and *D* to prevent slipping.

By this device an air-ether mixture can be blown down tube *A* under 2 to 4 cm. Hg pressure and carried to 1 cm. above the bifurcation of the trachea by tube *G*. Exhaust gases pass between the inner and outer tubes and out through *D*. The latter is connected with a metal tube which can be submerged (thus regulating the exhaust pressure) to the desired level. Pulmonary inflation to secure *apnea vagi* can be obtained easily by raising the exhaust pressure. This manipulation allows one to abolish *all* thoracic movement. Restoration of "negative" intrathoracic pressure is accomplished by lung distention at time of closure of the thoracotomy.

SUMMARY

An intubation tube for use with positive pressure apparatus for anesthesia in dogs undergoing intrathoracic surgery, together with its method of use, is described.

REFERENCES

1. Markowitz, J.: Textbook of Experimental Surgery, Baltimore, 1937, William Wood and Co., p. 23.
2. Hamilton, W. F., Woodbury, R. A., and Vogt, E.: Differential Pressures in the Lesser Circulation of the Unanesthetized Dog, Am. J. Physiol. **125:** 130, 1939.
3. Livingstone, H., and Hrdina, L. S.: A Modified Meltzer Apparatus for Anesthesia in Animals, J. Lab. & Clin. Med. **16:** 74, 1930.

A SIMPLE AND COMPACT ERGOGRAPH FOR STUDENT USE*

G. L. Maison and A. G. Broeker, St. Louis, Mo.

THE instrument to be described has several advantages over ergographs in common use at present. It is readily stored, having over-all dimensions of 18 by 6½ by 4 inches. It permits recording of excursions on drum. It records on an automatic counter the total distance through which force is applied. It can be used with unlimited stroke, or the length of stroke can be fixed so that uniform strokes are made. Its principal disadvantage is that experiments demonstrating the effect of varying the load on muscular ability can be done only if several machines of different inherent resistance are available for the student to use. The considerable variety of experiments which can be performed with fixed load make the machine useful despite this disadvantage.

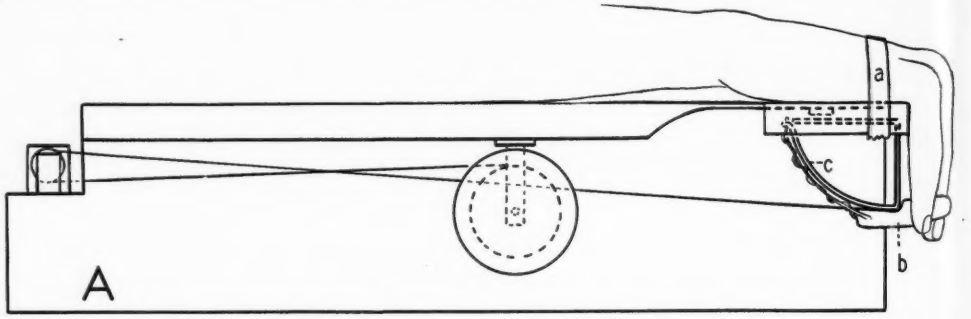
The Ergograph.—Fig. 1*A* shows a simple sketch of the machine with fingers in place ready for use. Note that the muscle used is the *extensor digitorum communis.* A consideration of the anatomy of the forearm and hand will show that with the metacarpus firmly fixed to the board by strap (*a* in Fig. 1*A*) no other muscle can apply force to the work.

The fingers are attached to the instrument by means of a rawhide thong. Fig. 1*A* shows the instrument seen from the side (sideboard removed) with the fingers in place in the thong *(b)*. Fore, middle, and ring fingers are inserted. Connection is made from the thong by means of wire to the furthest point of a metal quarter circle (*c* in Fig. 1*A* and *B*). This piece is made of sheet brass 0.036 inch thick, 2 inches wide, and 4 inches long, bent to a right angle (Fig. 2*A*) to which is soldered a piece of the same sheet brass curved to a radius of curvature 2 inches. The result seen from the side is a quarter circle (Fig. 2*B*). The wire is attached at the uppermost end of the curved sheet. The center of the quarter circle is now soldered to a brass rod 6 inches long and 3/16 inch in diameter at about its center (Fig. 2*C*). This forms the axis to which force is applied. The ends of this rod are drilled out to receive a piece of hardened steel rod ⅛ inch in diameter which is allowed to project from the end of the rod to act as a bearing (Fig. 2*D*). These bear on holes of proper size drilled in side pieces of brass rectangular bar ⅛ by ¾ inch which are recessed and screwed to the sides of the top board of the ergograph. The load factor or resistance to the movement of the axle is supplied by a coiled music wire spring whose lumen diameter is about ¼ inch.† It is slipped over the axle and anchored to the brass quarter circle on one end, and to the bearing plate on the other (*d* in Fig. 1*B*). Friction between the axle and the spring is prevented by having the lumen diameter of the spring greater than the diameter of the axle and also by soldering a small brass collar (*a* in Fig. 2*E*) to the bearing plate within which the axle fits and on the outside of which the spring rides. The resistance

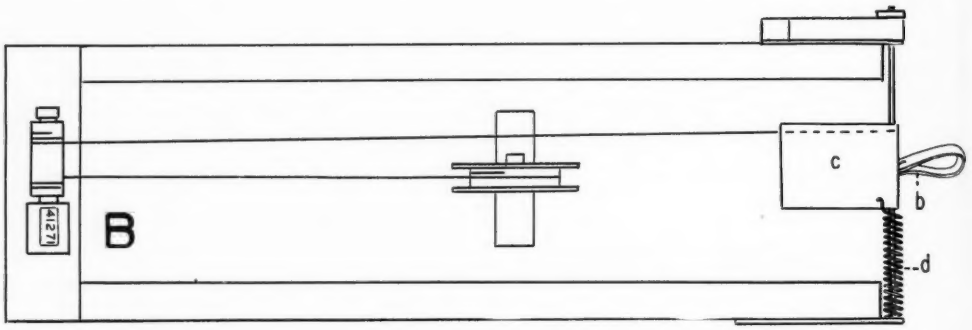
*From the Department of Physiology, St. Louis University School of Medicine.
Received for publication, March 6, 1940.

†Music wire 0.048 inch in diameter fashioned into a spring of ¼ inch inside diameter having 23 turns in 2 inches length tautened by one full turn before being anchored produces about 800 Gm. tension, which is a good resistance for untrained extensor digitorum communis muscles.

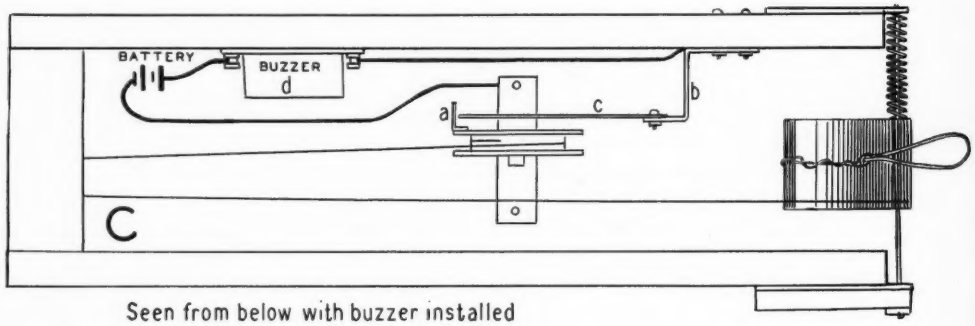
to turning of the axle will depend on the strength of the spring wire used and on the number of turns made on it before it is anchored. The finger thong is attached around the circle so that the force will always be applied at a fixed distance from the axis.



Seen from side, as if side board were transparent



Seen from above, as if top board were transparent



Seen from below with buzzer installed

Fig. 1.

The same principle is used in activating the meter to count the number of centimeters through which the force is applied. A small hole is drilled in the anterior end of the curved brass plate and a cord is fixed in this hole (Fig. 2*F*). The cord runs backward under the arm board to a spindle to which it is anchored after several turns. The spindle is 5 cm. in circumference and 1.5 inches long. It is held up on one side by a brass bearing and on the other by the shaft of a Veeder rotary ratchet counter (type Q13) to which the spindle is fixed. Each rotation of the spindle will turn the counter through 10 units and will imply that force has been applied through 5 cm. Thus each unit on the counter is 0.5 cm. Since turning the axle pulls the cord over an arc at a constant distance from the axis of rotation of the axle, the distance indicated by the counter will be correct.

After a movement the axle will be returned to normal position by the spring. The cord must be returned by a separate mechanism. This is accomplished by applying a second cord to the spindle which after several turns passes forward under the arm board to a spring wheel (Fig. 2*G*) which exerts a tension tending to unwind the cord from the spindle. Thus when the axle is turned, the cord is wound off the left side of the spindle, activating the counter. Simultaneously, the cord is also wound on the right side of the spindle from the spring wheel. After the force is released, the spring returns the axle to place, freeing the axle cord to the spindle. The tension of the spring wheel brings the cord back from the spindle onto the wheel and thus returns the axle cord to the spindle. The counter is prevented from backward rotation by its inherent ratchet.

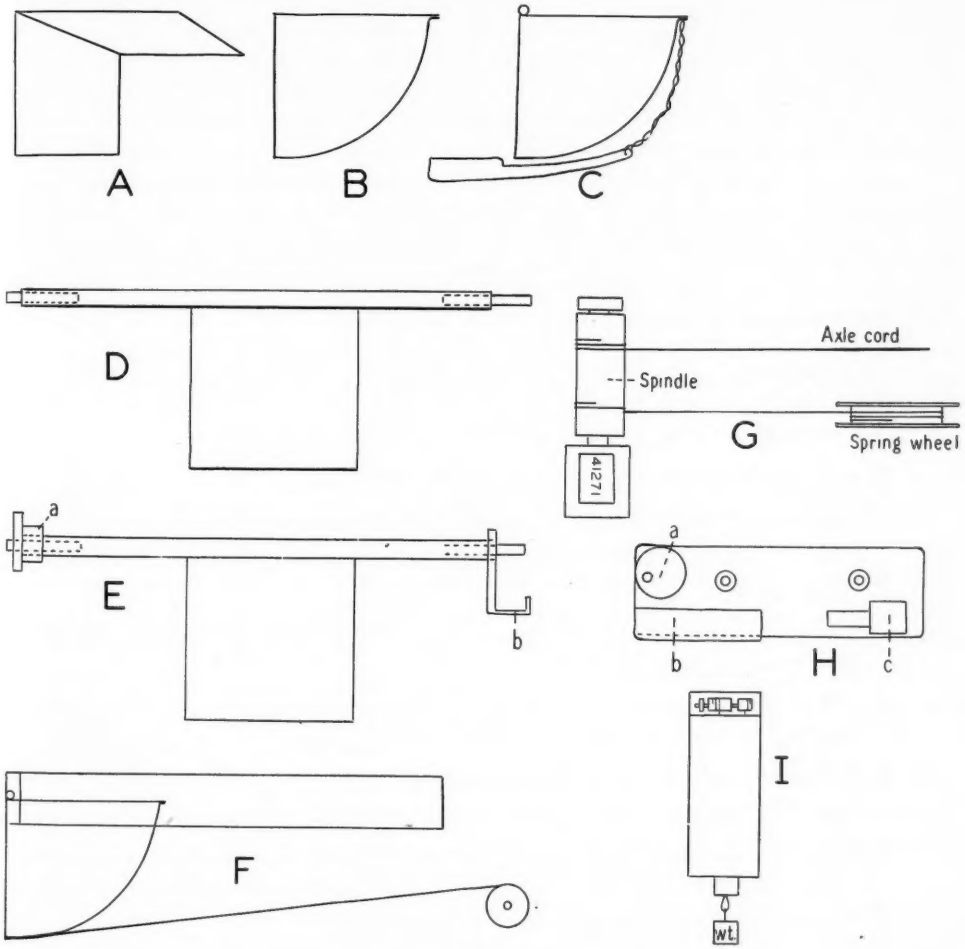


Fig. 2.

The ergograph is designed to record on drum through air transmission to a tambour with fall-away lever. The hardened steel bearing peg inserted in the shaft on the side opposite the spring is made long enough to extend ½ inch beyond the bearing plate (see Fig. 2*D* and *E*). To this peg is fixed an eccentric cog ½ inch in diameter and ¼ inch thick (*a* in Fig. 2*H*). Below this cog, and fixed to the bearing plate, is a brass trough (*b* in Fig. 2*E* and *H*) which lies ⅛ inch below the base of the eccentric cog when the axle is twisted to its maximum stroke. The trough is 5/16 inch wide. A rubber tube is inserted between the cog and trough. At the posterior end of the trough there is soldered to the brass plate a brass rod projection (*c* in Fig. 2*H*) upon which the rubber tubing can be

fixed so that it is firmly held and prevented from leaking air. The other end of the rubber tube is attached to the tambour. In actual use the strokes upon the drum can be readily translated into centimeters of excursion by calibrating the extent of excursion per given distance as measured on the counter.

For certain applications it is desirable to make the ergograph strokes uniform. This is readily accomplished by arranging contacts so that a buzzer sounds when the stroke reaches a given distance. Fig. 1*C* shows contacts and buzzer installed. A brass rod ½ by ¼ by ⅛ inch is soldered to the outer edge of one flange of the spring wheel so that it projects radially (*a* in Fig. 1*C*). To the undersurface of the arm board there is affixed a metal bar *(b)* which acts as a base for a swing lever *(c)* of brass 0.025 by 0.125 by 4.0 inch. The swing lever is prevented from downward swing by crimping its end behind the fulcrum, and prevented from upward swing by a simple spring wire attached to the fulcrum. Thus it is held horizontal ½ inch below the undersurface of the sideboard. An electric door buzzer *(d)* is fixed to one of the sideboards. A dry cell is connected to one pole of the buzzer and to the base of the spring wheel. The second buzzer pole is then connected to the swing lever holder.

When the fingers are extended, the spring wheel is rotated until the projecting contact strikes the swing lever. The circuit is then completed, ringing the buzzer. The subject pulls until the buzzer sounds, relaxes, and pulls again, making uniform strokes. If the buzzer is disconnected, or if stroke is continued despite the buzzing, nothing prevents the wheel from rotating as the spring of the swing lever readily gives. Thus the buzzer can be left out of operation by simply disconnecting the battery.

The advantage of stroke uniformity is that it establishes a neat end point to call fatigue. Fatigue can be defined as that condition of the person in which he cannot make a stroke of this length in the given time interval.

Some Applications of the Ergograph for Student Use.—

1. Demonstration of treppe—human fatigue curves.
2. Comparison of work a muscle can do circulated with the work it can do when ischemic.
3. Effect of rate of contractions on total work ability of muscle, (a) circulated or (b) ischemic.
4. Effect of motivation on muscular ability—comparison of work output ringing buzzer with work output without buzzer.
5. Effect of massage on muscular ability.
6. Effect of fatigue of other muscles on the work of an unused muscle.
7. Effect of ischemic work on the blood pressure and heart rate (reflex of Alam and Smirk).
8. The character and properties of ischemic pain.

(All these experiments except No. 4 can be done with or without the buzzer.)

Calculation of the Work Done on the Ergograph.—The resistance against which the force works is that of a spring. This means that the resistance increases gradually throughout the stroke. The resistance can be calibrated by hanging weights on the finger thong with the ergograph suspended in a vertical position (as in Fig. 2*I*). Gravity acting on the weights will overcome the resistance of the spring. If gravity acting on a weight of 1,100 Gm. moved the counter 4 units (2.0 cm.), then $1{,}100 \times 2.0 = 2{,}200$ gram centimeters of work

must be expended every time a stroke 2.0 cm. long is made. On a particular machine the following calibration was obtained:

Weight Suspended	Average Movement Recorded
1,100 Gm.	1.3 cm.
1,150 Gm.	2.8 cm.
1,200 Gm.	3.5 cm.
1,250 Gm.	4.5 cm.
1,300 Gm.	6.8 cm.
1,350 Gm.	9.0 cm.

This implied that a stroke of 6.8 cm. required the same work as would have been required if a constant load of 1,300 Gm. had been used, and that $6.8 \times 1{,}300 = 8{,}840$ gram centimeters of work were required to make the stroke of this length. For student use a simple method of calculation was desired. With the buzzer in use there was no difficulty, since all strokes were uniform and gravitational calibration gave the uniform load equivalent of this stroke. Thus the work was calculated as the product of distance times this equivalent. When the buzzer was not being used and the length of the stroke varied widely, it was questionable whether a gravitational equivalent based on average stroke length would be sufficiently accurate. The average stroke length in any given work bout was readily obtained by dividing the distance recorded on the counter by the number of strokes made by the subject. The number of strokes can be counted as made or from the kymogram. Several methods of calculation suggested themselves and were checked on twenty different records made on various experimental procedures.

Method 1. From the twenty different trials the average stroke for all trials was 6.4 cm. Referring back to the calibration and interpolating, the uniform load equivalent was about 1,290 Gm. Multiplying the distance factor in each bout of work by 1,290 then approximated the work in gram centimeters.

Method 2. Another possibility was to use the average stroke for each particular bout of work. In the twenty different trials the average stroke varied from 5.1 to 7.8. The distance in the bout of 5.1 cm. average stroke was multiplied by 1,260 Gm. to obtain the work; the distance in the 7.8 bout was multiplied by 1,320 Gm. to obtain the work.

Validification of Method.—Both methods 1 and 2 arrive at approximations, and while it is obvious that the work results by the two methods can show no greater variation in these twenty records than 3 parts in 129, one has no idea how far the result by either method is from the true work done.

Method 3. The work done in each bout was determined accurately as follows: Before each of the twenty work bouts a calibration of centimeters of stroke against millimeters of tambour excursion was made. This is rendered easy by the automatic counter, since by watching the counter, strokes of 1 cm., 2 cm., 3 cm., etc., can be made. After the record was fixed, each stroke was translated from millimeters to centimeters so that an incidence record resulted. Table I shows the incidence of different strokes in a particular record. It can be seen that the strokes varied from 2.25 to 8.0 cm. in length. Referring back to gravitational calibration, the gravity equivalent of each one of these strokes was obtained as seen in the fourth column of Table I. Now the centimeter equivalent was multiplied by the incidence of such strokes, and the product was

multiplied by the gravity equivalent of this length of stroke. The sum of these results gave the work done. The work calculated by methods 1 and 2 in this case was 498 × 1,290 = 643,000 (1) and 498 × 1,295 = 646,000 (2).

TABLE I

CALCULATION OF WORK BY INDIVIDUAL STROKES

(Incidence From Tambour Record)

MM.	CENTIMETER EQUIVALENT		INCIDENCE		GRAVITATIONAL EQUIVALENT		
11	8.0	×	2	×	1,325	=	21,200
10	7.4	×	21	×	1,310	=	204,000
9	6.75	×	34	×	1,300	=	298,000
8	6.25	×	9	×	1,290	=	72,500
7	5.75	×	1	×	1,275	=	7,300
6	5.10	×	1	×	1,260	=	6,400
5	4.5	×	3	×	1,250	=	16,900
4	4.0	×	2	×	1,225	=	9,800
3	3.4	×	2	×	1,200	=	8,200
2	2.25	×	2	×	1,125	=	5,100
							649,400

One may wonder whether the ability to measure tambour strokes on a record is good enough to warrant calling method 3 accurate. There is a ready check on the accuracy of the measurement as follows: If in Table I the centimeter equivalents are multiplied by the incidence alone and all the products are summed, the result is the total distance through which the force was applied; in this case the sum was 500.9 cm. But the counter also added up the total distance during the work bout as 498 cm. Thus the measurement is checked.

This procedure was carried out with each of the twenty records, and the percentage error of work as calculated by methods 1 and 2 from method 3 taken as accurate varied from –3.9 per cent to +2.1 per cent for method 1; from –3.1 per cent to +1.7 per cent for method 2. We conclude that the error involved by either method 1 or 2 is small; that method 2 is somewhat less subject to error than method 1.

For student use we furnish a weight calibration with each instrument and instruct the student to use method 2. After the instrument is assembled it requires a week or two for the spring to take its set. The force of the spring seems to remain quite constant once the set is taken. Probably gravitational calibration at least once a year will be necessary.

SUMMARY

A student ergograph for human extensor digitorum communis has been described. Its over-all dimensions are 18 by 6½ by 4 inches. It is economical to construct. It permits limitation of stroke length so that fatigue can be more sharply defined as an end point. A list of experiments to which it can readily be applied is given. Methods available for calculation of the work done on the machine are discussed and validified. The portability of the ergograph recommends it for clinical use in following the work ability of patients under treatment for Addison's disease, and myasthenia of any other origin in which the various muscles are fairly uniformly affected.

THE USE OF A SINGLE GUINEA PIG FOR VIRULENCE TESTING*

MARGARET BEATTIE, M.A., GR.P.H., BERKELEY, CALIF.

IN A PREVIOUS study[1] it was demonstrated that the use of a single rabbit for virulence testing by means of the coincident intracutaneous injection of a test and a control suspension of *C. diphtheria* culture mixed with antitoxin (instead of the parallel injection of the culture suspension into an unprotected and antitoxin protected animal) so enhanced the inherent variability of rabbit reactivity to diphtheria toxin as to yield falsely negative reactions in tests with known pure cultures of *C. diphtheriae.* This modification of the traditional technique was introduced by Fraser, Halpern, and Roy[2] as an economical way to test the large number of cultures involved in research or survey investigations. Only under extraordinary circumstances would a public health laboratory have so many tests to perform at one time or have rabbits available for this purpose. In such a laboratory the economy involved in the use of a single animal for a few tests would be desirable. In health department laboratories it is important to discover immediately whether organisms, with morphology characteristic of *C. diphtheriae,* when isolated from the throats of carriers, recovered patients, or immunized contacts, are virulent. The infectiousness of the immunized child whose throat lesion yields organisms morphologically resembling diphtheria bacilli is also important to health authorities.

At the time the experiments on rabbits were done, a few guinea pigs were tested by this technique. These tests indicated that while the guinea pig was less apt to vary in reactivity to the injections than the rabbit, some differences in reaction occurred in animals injected with control suspensions. In order to limit the effect of antitoxin, the number of tests to be placed on each animal was reduced from eight to four. The amount of antitoxin in the control suspension was decreased to what appeared to be the least amount for neutralization of toxin.

Cultures.—Four cultures were selected from the sixteen which had been used on rabbits. One (No. 75), a gravis strain, was chosen as a control culture because it had reacted positively on all rabbits and guinea pigs. Two intermedius strains (Nos. 133 and 198) which had frequently yielded inconclusive or negative reactions were included as test strains. The fourth test culture was a mitis strain (No. 206) which had occasionally induced negative or inconclusive reactions on rabbits.

Test Inoculum.—In order to obtain a sufficient amount of culture suspension, several slopes of Loeffler's medium were inoculated with each test culture. This was accomplished by delivering with a pipette a saline suspension made

*From the Department of Hygiene, University of California, Berkeley.
Aided by a grant from the Research Board, University of California.
Received for publication, March 11, 1940.

from the growth on a Loeffler's slant onto the surface of each of several slopes. Complete seeding of the surface was accomplished by rotating the inoculum over the surface of the slopes before placing the tubes in the incubator. After forty-eight hours' incubation the growth on each slope was suspended in 2 c.c. of Stone's suspending solution.[3] A stained smear from each test culture was examined to check the presence of morphologically typical bacilli in pure culture. Subcultures were made from one tube of each set of cultures to be certain the material was viable. Because of the color and consistency of the suspending solution, only a rough estimation of the relative opacity of the suspensions could be made.

Control Inoculum.—To each cubic centimeter of suspension prepared as described above, 0.1 c.c. of antitoxin, containing approximately 100 units, was added. The suspension was well mixed and allowed to stand at room temperature for at least half an hour before being injected. This amount of antitoxin was evidently near the minimum because very slight necrotic reactions were occasionally observed.

Animals.—Of 129 guinea pigs all were white females except eleven which were white males. All animals were fully mature and weighed more than 400 Gm. though they varied considerably in weight, size, age, and skin texture.

THE EXPERIMENT

Five groups of from twelve to forty animals were tested within a three months' period. In each experiment the sex and size of the animals were considered in selecting those to be compared as test and control animals. Test animals received intracutaneous injections of both test and control culture suspensions coincidentally. Control animals received injections of test culture suspensions only. Each animal received the same culture suspension in the same skin area for any experiment. One-tenth cubic centimeter of inoculum was injected intracutaneously into the shaven skin on the side of guinea pigs. The control animals received 1,000 units of antitoxin five hours after the tests were placed. Both test and control animals received 1,000 units of antitoxin twenty to twenty-six hours after the tests had been placed. The reactions were observed at twenty-four-hour intervals for three days. Reactions were designated as positive when an area of necrosis resulting in a scab more than 4 mm. in diameter was formed; inconclusive when a small scab (less than 4 mm.) was observed; and as negative when no area of necrosis was noted.

RESULTS AND DISCUSSION

One hundred and twenty-nine guinea pigs were injected. Sixty-five guinea pigs (test animals) were injected with both test and control culture suspensions. Sixty-four guinea pigs (control animals) received injections of test suspension alone. Only one culture (No. 198) induced inconclusive and negative reactions which indicated variation of reactivity in the guinea pigs tested.

Of the sixty-five test animals, 53 reacted positively to all injections. Five animals had inconclusive reactions and seven gave negative reactions at the site of the injection of one test culture (No. 198).

Sixty-three of the 64 control animals yielded positive reactions to all test injections. One animal, on which all reactions developed slowly and were small, showed an inconclusive reaction at the site of injection of one test culture suspension (No. 198).

Differences in time and rate of development and size of lesion were apparent in different animals, but, on the whole, the lesions on the test animals were obviously smaller, particularly the lesions induced by the culture (No. 198) which was the sensitive indicator of any variation in guinea pig reactivity. The diameter of the scabs was measured in one group of 40 animals in order to verify this apparent difference. Culture No. 198 on the control group of guinea pigs induced scabs whose average diameter measured only 0.2 mm. less than the smallest average scab measurement of the three other cultures tested, whereas in the test series the scabs induced by this culture had an average measurement 2.0 mm. less in diameter than the other cultures. These measurements completely confirmed the observation that the reactions were larger on the animals of the control series.

It is evident that the use of control inocula containing antitoxin modifies the reaction on single animals injected intracutaneously for diphtheria virulence testing so as to render erroneous results. It might be possible to limit the application of the technique to one or two tests by determining the maximum amount of antitoxin which could be safely used, but this would involve the use of antitoxin closely measured for maximum as well as for minimum unit content. Such antitoxin is not available in the public health laboratory. A single animal can be utilized by repeating the injection of the test suspension in another skin area of the animal converted into a control by the intravenous injection of antitoxin five hours after the test injections are placed. This modification of technique was described by Fraser and Weld in 1926.[4] The time interval and the labor involved in repetition of the injections frequently complicates the schedule so as to render the test inconvenient in a public health laboratory.

CONCLUSIONS

Guinea pigs vary only slightly and much less than do rabbits in their reactivity to the injection of cultures of *C. diphtheriae*. The coincident intracutaneous injection of a control mixture of culture and antitoxin which permits the use of a single guinea pig for several tests cannot be recommended since false negative or inconclusive reactions may occur.

REFERENCES

1. Beattie, Margaret: The Use of a Single Animal for Testing the Virulence of C. diphtheriae, J. Lab. & Clin. Med. **25**: 1111, 1939.
2. Fraser, D. T., Halpern, K., and Roy, I. S.: Studies in Diphtheria, Canad. Pub. Health J. **29**: 40, 1938.
3. Stone, R. V., and Weigel, C.: A New Suspending Medium Used for the Intracutaneous Virulence Test of Bacillus diphtheriae, Am. J. Pub. Health **9**: 113, 1929.
4. Fraser, D. T., and Weld, C. B.: The Intracutaneous Virulence Test for Corynebacterium diphtheriae, Tr. Roy. Soc. Canada **20** (sec. V): 343, 1926.

ON THE PRESERVATION OF SPECIFIC ANTI-M AND ANTI-N AGGLUTININS WITH TOLUOL*

PHILIP LEVINE, M.A., M.D., NEWARK, N. J.

IT IS well known that antisera, when preserved in the cold in the filtered state, retain their antibody content almost undiminished over a period of several years. For the production of diagnostic anti-M and anti-N reagents, it is necessary to remove the species-specific agglutinins by absorption with suitable bloods lacking the factors in question. For this purpose, the serum in a dilution of 1:15, 1:20, or higher is absorbed for one-half to one hour with one-half its volume of washed blood sediment. Generally, one absorption suffices to remove the species-specific agglutinins, but occasionally a second treatment, with perhaps a smaller quantity of blood, may be required. While these manipulations can be carried out under sterile conditions, this is not convenient, and consequently the diagnostic fluids become unsuitable for use after a few days in the ice chest because of bacterial contamination. Once potent diagnostic reagents are prepared, it is of considerable advantage to preserve them for future use on account of the large quantity of selected blood necessary for the absorption and the time and effort required for the numerous manipulations.

A number of years ago I[1] tested a few preservatives and observed that the addition of 1 drop of toluol per cubic centimeter of diagnostic fluid, stoppered and sealed with paraffin, gave very good results. Tests with diagnostic fluids preserved in this manner for a period of six to twelve months have invariably given unequivocal results, so that the tests always gave a sharp distinction between bloods possessing and bloods lacking the agglutinogens in question. Those rare bloods reacting weakly with potent diagnostic fluids, freshly prepared, were invariably agglutinated by the preserved fluids. At no time in an active, continued experience, extending over a period of ten years with the use of fluids preserved in this manner, were any difficulties encountered in the diagnosis of the M and N factors.

In a study of various preservatives of diagnostic fluids, Boyd[2] comes to the conclusion that there are a number of preservatives (such as brilliant green or acriflavine) which are preferable to toluol. However, Boyd made no tests with toluol preserved fluids before four and one-half years elapsed, at which time all activity had disappeared.

For this reason, I made tests with anti-M and anti-N diagnostic fluids preserved with toluol for a period of thirty-two months. These were the only fluids available to test the efficacy of the preservative, but it seems to me that a preserved diagnostic fluid still potent after an interval of thirty-two months in the ice chest is certainly properly preserved.

*From the Division of Laboratories, Newark Beth Israel Hospital, Newark.
Received for publication, March 13, 1940.

Actually, these specific diagnostic reagents were found to give potent reactions that compared favorably with those obtained in simultaneous tests with freshly prepared reagents from the same antisera (Tables I, II, III).

TABLE I

DIAGNOSTIC FLUID	GROUP TYPE	O M	A M	A M	A MN	A MN	A MN	B MN	O N	A N	A N
Anti-M (1)		++++	++++	++++	++++	++++	++++	++++	0	0	0
Anti-M (2)		++++	++++	++++	++++	++++	++++	++++	0	0	0
Anti-N (3)		0	0	0	+±	+±	++	+++	++++	++++	++++
Anti-N (4)		0	0	0	++	++±	++±	+++	++++	++++	++++

The plus signs indicate varying degrees of agglutination.
(1) Prepared from serum 136, diluted 1:15, and preserved with toluol on July 3, 1937.
(2) Freshly prepared from serum 136, diluted 1:15.
(3) Prepared from serum 52, diluted 1:20, and preserved with toluol on July 3, 1937.
(4) Freshly prepared from serum 52, diluted 1:20.

TABLE II

TITRATION OF THE ANTI-M FLUIDS

		1:6	1:12	1:24	1:48	1:96
Anti-M No. 136 preserved for 32 months	(1) OM	++++	+±	+	tr	0
	(2) AM	++++	+++	+	0	0
	(3) OMN	++++	+++	+	0	0
	(4) AMN	++++	+±	±	0	0
Anti-M No. 136 fresh	(1) OM	++++	+±	+	0	0
	(2) AM	++±	+	0	0	0
	(3) OMN	+++±	+±	tr	0	0
	(4) AMN	+±	+	0	0	0

tr. indicates trace.

TABLE III

TITRATION OF THE ANTI-N FLUIDS

		1:6	1:12	1:24	1:48
Anti-N No. 52 preserved for 32 months	(1) ON	+±	+	tr	0
	(2) AN	+±	+	f.tr.	0
	(3) OMN	±	0	0	0
	(4) AMN	+	±	0	0
Anti-N No. 52 fresh	(1) ON	++±	+	f.tr.	0
	(2) AN	+±	+	tr	0
	(3) OMN	+	tr	0	0
	(4) AMN	+±	+	0	0

tr. indicates trace; f.tr., faint trace.

It is clear that the anti-M diagnostic fluids, preserved with toluol for thirty-two months, is just as active as the fluid freshly prepared from the same serum; the preserved anti-N fluid gave reactions which were only slightly less intense than those with the freshly prepared fluid. Titrations of the fresh and preserved anti-M fluid gave results which indicate that the preserved fluid is, indeed, somewhat stronger than the freshly prepared reagent. Similar slight differences of behavior of the two diagnostic fluids were observed in testing with other bloods, not recorded in Table II. The titration of the anti-N fluids gave results which confirm those of Table I, namely, a slightly greater activity on the part of the freshly prepared fluid.

Obviously, there are other suitable chemical preservatives for specific anti-M and anti-N reagents, as has been shown by Boyd. Another suitable procedure would seem to be drying with some such technique as the Flosdorf-Mudd apparatus. In any event, the results here reported indicate that toluol may be just as efficient a preservative as, for instance, acriflavine, especially since Boyd's anti-M fluid No. 887, preserved for twenty-two months with acriflavine, already showed a considerable diminution in activity.

Addendum: Retest of the same diagnostic fluids on January 20, 1941, i.e., after preservation of three and one-half years, showed no loss of activity in the anti-M reagent. The anti-N reagent suffered a drop of titer, distinct reactions obtainable at a dilution of 1:6 instead of 1:12; tested in the undiluted state, the anti-N diagnostic fluid gave clear-cut differentiation.

REFERENCES

1. Levine, Philip: Unpublished experiments.
2. Boyd, W. C.: Production and Preservation of Specific Anti-Sera for Blood-Group Factors A, B, M, and N, J. Immunol. 37: 65, 1939.

A SIMPLE, INEXPENSIVE, VERTICAL ILLUMINATOR FOR THE STUDENT MICROSCOPE*

ALRICK B. HERTZMAN, PH.D., ST. LOUIS, MO.

THE need for vertical illumination for examining human skin capillaries and minute vessels in the frog in the student laboratory directed attention to the possibility of using for this purpose the very effective pencil flashlight bulb known as Mazda 222 (operating on 2.2 volts). A simple, flexible, convenient, and effective mounting was provided with a dial socket, screw type (costing four cents), carried by a copper wire, 10 inches in length and ⅛ or ¼ inch in diameter. This in turn was clamped to the ring stand. The beam of light could then be directed to the vascular area to be observed. The arrangement can be brought close enough to provide enough light for observation of the minute vessels unless high magnifications are required. When strong illumination is desired for microphotography, the bulb may be operated momentarily above its rated voltage by inserting a single pole double throw toggle switch in the lead to the bulb and providing a source of slightly higher voltage.

The current source may be dry cells, but in the student laboratory we operate a group of bulbs on one filament transformer supplying a.c. at 2.5 volts. A short piece of resistance wire in the lead to the bulb reduces the voltage to the correct value. When the bulbs are operated below their rated voltage, they last twenty-four hours or more in continuous operation.

We placed thirty of these illuminators in the student laboratory at a cost of less than ten dollars. They proved very satisfactory for student study of the minute vessels. Other uses will suggest themselves.

*From the Department of Physiology, St. Louis University School of Medicine.

THE USE OF DIAPHANE FOR MOUNTING GIEMSA TYPE PREPARATIONS*

FREDERICK COULSTON, M.A., SYRACUSE, N. Y.

IN LABORATORIES where Romanowsky-type stains are used, it has always been a problem to find a satisfactory as well as permanent mounting medium. This is especially true of those laboratories where malaria research is done. For here the routine method of staining the parasites invariably consists of the use of one of the better Romanowsky stains, such as Giemsa's, MacNeal's tetrachrome, or Wright's.

In general, three types of preparations are used; namely, blood smears, and smears and sections prepared by dehydration. When these are mounted in balsam, the stain almost always fades considerably within a few months or a year. This fading is usually found in the region of the smear or sections farthest from the edge of the cover slip, leaving a rim of fairly well-stained material. The reason advanced for this gradual destaining process is that the α and β abietinolic acid in the balsam gradually oxidizes, thus changing the pH of the medium. And since the Romanowsky stains work best in an alkaline state, this causes them to fade.

Many alterations in the balsam have been suggested to counteract this tendency toward acidity. In Lee's[1] *Vade Mecum* (1928, p. 231) a method of making neutral balsam is given which consists essentially of mixing the balsam crystals with an equal amount of sodium bicarbonate. Scott[2] (1911) recommends placing a piece of marble in the balsam bottle and protecting the bottle from light. A similar suggestion is made by Guyer[3] (1936, p. 129). This works satisfactorily for awhile but fading eventually occurs. Cannon and Taliaferro[4] (1931) mounted sections in balsam without cover slips, stating that this prevented rapid decolorization. Huff and Bloom[5] (1935) used a petrol-ether soluble fraction of crude dammar dissolved in xylene as a mounting medium. Kingsley[6] (1937) recommends keeping stained megakaryocytes and platelets in sections mounted in neutral xylene dammar away from light. Lee (p. 503) suggests mounting Giemsa-stained sections in cedar oil rather than in balsam, since in the latter the colors fade more rapidly.

In 1927 Wright[7] showed that euparal was an excellent mounting medium for blood smears stained with the many Romanowsky-type stains. Slides so mounted did not fade for over six years. Euparal was introduced by Gilson[8] (1906) who stated it was a mixture of eucalyptol, paraldehyde, and camsal. A similar American product is sold by some supply houses under the name of diaphane. This product is said to contain euparal, ether, gummed sandarac, and paraldehyde. Shepherd[9] (1918) presented the formula of a substitute for euparal which he claimed was in all respects similar to it. Both euparal and diaphane have a refractive index of 1.483. Each is available in a colorless and a green form. The latter contains a copper salt, the color of which is said to

*From the Department of Zoology, Syracuse University, Syracuse.
Received for publication, March 16, 1940.

intensify hematoxylin stains. The colorless diaphane is the proper one to use for mounting Romanowsky-type stained preparations. Histologic comparisons of diaphane and euparal indicate that they are identical in properties. Therefore, in this paper when reference is made to diaphane, it is implied that the same is true of euparal.

Diaphane is superior to balsam in many respects, and especially in that it is a nonoxidant and is neutral to all stains. It also has a lower index of refraction, enabling observation of objects totally invisible in balsam or dammar. Most materials may be mounted in it directly from 95 per cent alcohol.

Recently, Groat[10] (1939) described two new cycloparaffin mounting media, commercially called Nevillite V and No. 1. These substances are similar to balsam and are soluble in toluene and xylene. They are said to be superior to balsam in that they do not become acid with age. However, they have higher refractive indices (Nevillite V, 1.544; Nevillite No. 1, 1.567) than balsam (1.535), which makes them optically much inferior to diaphane (1.483). Furthermore, Nevillite must be used with absolute alcohol and a clearing agent such as xylene.

MATERIALS AND METHODS

Two simple and efficient techniques were developed permitting the use of diaphane as a mounting medium after staining with Giemsa's, Wright's, Jenner's, and the Eosin Azure II method of Maximow's. In general, the following discussion applies specifically to preparations stained with Giemsa's, since it is used routinely in this laboratory.

The first technique is to be used with alcohol-acetone mixtures, and the second with dioxane as the dehydrating agent. Both give approximately the same results. The advantage of the one method over the other depends on the circumstances in which they are to be used. For ordinary work, or when it is difficult to obtain dioxane, the alcohol method will be most convenient and inexpensive. Since absolute alcohol is not necessary, it will be found especially valuable for work in humid climates, such as the tropics. The dioxane method gives advantages familiar to all who have had occasion to use dioxane.

The staining technique employed was essentially a simplification of Wolbach's[11] (1919) method for staining paraffin sections with Giemsa. We have used the technique in staining smears and tissue preparations of bird malaria, Hemoproteus, and fowl pox. Malaria and Hemoproteus parasites stain blue in contrast to the red blood cells which stain a light yellow orange. The general cellular substance takes a rich blue appearance. In fowl pox, the Borrel and Bollinger bodies stain bright red against a blue background. A good account is given in Lee (p. 503) with many helpful suggestions. Hewitt[12] (1939) devised a modification of the Wolbach method which gives fine results for malaria parasites. However, this method is time-consuming and the dehydration and clearing procedure may be much abbreviated when diaphane is used instead of the cedar oil or neutral balsam recommended.

STAINING PROCEDURE

A. *Dry blood smears* were made in the usual manner by running a small drop of blood on the end of one slide along another. The smears were air-dried

and then fixed in absolute methyl alcohol (reagent). They were then allowed to dry and stained with a mixture of 2 drops of the stock Giemsa to each cubic centimeter of water used. After ten to thirty minutes they were removed, washed, and dried. If the slides are to be mounted, diaphane may be applied directly to the dry smear covering with a No. 1 cover slip.

B. *Smears and tissues prepared by dehydration* were always fixed in Maximow's Zenker-formol (10 per cent formalin; no acetic acid). Preparations were immersed in this fluid immediately to avoid drying. Blood or tissue smears were fixed for thirty minutes or less; small pieces of tissue for two to three hours.

(1) *Smears* were washed in water (running preferred) for at least five minutes. If *alcohols* are to be used, pass through to 70 per cent alcohol slightly colored with iodine. Leave for ten to fifteen minutes and remove. Pass smear back through the alcohols to water. Mordant with a 2.5 per cent solution of potassium bichromate for five to ten minutes. Wash and stain with a mixture of 4 drops of stock Giemsa to each cubic centimeter of water used. Usually 2 c.c. of fluid is sufficient to cover a slide completely. Ordinary tap water is satisfactory if slightly alkaline and low in mineral content. Allow blood smears to stain for at least thirty minutes, and tissue smears for as long as two hours. If *dioxane* is used it is not necessary to include the iodine step at all, since, as Guyer (p. 66) points out, dioxane dissolves bichloride of mercury. With dioxane the smear is passed through at least three changes of pure dioxane and then washed in water and stained as described. The mordant and stain may be conveniently applied by flooding the slides on a staining rack.

(2) *Tissues* dehydrated with *alcohols* were first washed for at least twelve hours in running water. They were then passed through the alcohols in the usual manner, including the iodine step, to absolute alcohol. Clearing and infiltration were done with toluene and absolute alcohol mixtures following the method of Galigher[13] (1934, p. 122). Imbed and section as usual. Sections should be cut as thin as possible, 4 to 10 microns. The mounted paraffin ribbons were gently heated over an alcohol flame until just melted. The slide was immediately placed into two changes of pure xylene and then passed down through the alcohol series to water. Mordant and stain as for smears. All preparations should be allowed to stain for at least one hour. It is advisable to allow the mordant to remain for at least thirty minutes; longer periods will do no harm. When *dioxane* is used for dehydration, the tissues are immediately placed in dioxane without any preliminary washing. Change the dioxane at least once and imbed in paraffin, using the procedure of Guyer (p. 65). The mounted sections were heated over an alcohol flame as mentioned above, and then passed through two changes of pure xylene into dioxane. Wash in two changes of dioxane and transfer to water. Mordant and stain as previously described.

MOUNTING PROCEDURE

A. The Alcohol-Acetone Method. All Romanowsky-type stains are soluble in alcohol; it is this fact that makes the use of diaphane directly from 95 per cent alcohol impracticable, since a certain amount of the alcohol is carried over to the mounting medium, thus continuing the destaining process. This

eventually ruins the slide in as short a time as a week. In order to obviate this difficulty, the following technique was devised. It applies to both smears and tissues prepared by dehydration.

After staining, the preparations are dehydrated in an alcohol series. Destaining, if necessary, is carried out in 70 per cent alcohol. When a redder color is desired, the tissues may be placed for a few seconds in 95 per cent alcohol to which eosin has been added (1 drop of 0.5 Gm. eosin in 100 c.c. of 95 per cent alcohol to 10 c.c. of alcohol). Transfer from 95 per cent alcohol to solution A. Solution A is a mixture of 30 c.c. of 95 per cent alcohol, 30 c.c. of pure acetone, and 5 c.c. of diaphane. It is readily miscible. Destaining by the 95 per cent alcohol is stopped considerably in this mixture, but the slide should not be left in it longer than a minute. Transfer to solution B. This is a mixture of 55 c.c. of acetone, 10 c.c. of diaphane, and about 1 c.c. of 95 per cent alcohol. When the acetone and diaphane are mixed, a milky white solution results. But as the alcohol is added it clears up completely and remains so indefinitely. The alcohol is conveniently added drop by drop until the desired clear solution is obtained. This small amount of alcohol does not in any way cause destaining. Mount in diaphane.

Slides so prepared and mounted have remained without any evidence of fading for over a year. Preparations may be passed through solutions A and B directly from 70 per cent alcohol without any apparent damage to the tissues. The reason one cannot go straightway from 95 per cent alcohol to acetone to diaphane is that the solubility reaction between these substances is so drastic that the tissues usually crack and are ruined. The adding of diaphane to solutions A and B prevents the tissues from drying out quickly, since acetone is so volatile. It also prevents the acetone from going into solution too rapidly with the diaphane under the cover slip. The use of acetone with Romanowsky stains may be objectionable, but Kingsley[14] (1937) showed that it was not detrimental to such stains.

Diaphane solvent, a preparation of secret formula marketed by some supply houses, may be used with equal success in solutions A and B in place of acetone. If desired, solutions of acetone and the solvent can be employed. Diaphane solvent was miscible in all proportions with 95 per cent alcohol, acetone, and dioxane. When preparations are placed in the undiluted solvent from 95 per cent alcohol, the mixing is rather slow and destaining is not stopped immediately. When the solvent is mixed in excess with 70 or 80 per cent alcohol, a milky white solution, similar to the one described for solution B, results. The addition of a small amount of 95 per cent alcohol clears this up. It is recommended, therefore, that if the diaphane solvent is to be used as the clearing agent, it should be prepared and employed as solutions A and B, substituting it for the acetone.

B. The Dioxane Method. Dioxane was found to be soluble in all proportions with diaphane. As would be expected, it does not destain preparations stained with any Romanowsky-type stains used. This makes it an ideal fluid in which to dehydrate such material for mounting in diaphane (or for that matter in balsam or cedar oil). The following procedure was utilized with much success:

Subsequent to staining, the slides are washed in water and destained, if necessary, in 70 per cent alcohol directly from the water wash. Fifty per cent alcohol works just as efficiently and has the advantage of being less drastic in action. Stop destaining by transferring quickly to pure dioxane. Pass through two changes of dioxane. Mount in diaphane. No clearing agent is necessary. However, if desired, graduated mixtures of diaphane and dioxane may be used to good advantage. Since the dioxane is not so volatile as acetone, it is best to remove some of the excess before mounting, thus allowing the slide to dry more quickly. Preparations mounted in this manner have the advantage over the alcohol-acetone method in that the destaining is stopped abruptly. This results in more uniformly stained tissues.

CONCLUSIONS

1. Diaphane is unconditionally recommended as a mounting medium for preparations stained with any Romanowsky-type stains in place of balsam or cedar oil.

2. An alcohol-acetone and a dioxane method suitable for dehydrating tissues stained with the Romanowsky-type stains are presented. Each method has been used successfully.

3. A simple method for staining tissues with Giemsa which has proved especially useful in bird malaria and fowl pox research is described.

REFERENCES

1. Lee, B.: Microtomist's Vade Mecum, Philadelphia, 1928, P. Blakiston's Son & Co.
2. Scott, S. G.: On Successful Double Staining for Histological Purposes, J. Path. **16**: 390, 1911.
3. Guyer, M. F.: Animal Micrology, Chicago, 1936, Univ. Chicago Press.
4. Cannon, P. R., and Taliaferro, W. H.: Acquired Immunity in Avian Malaria. III. Cellular Reactions in Infection and Superinfection, J. Prev. Med. **5**: 37, 1931.
5. Huff, C. G., and Bloom, W.: A Malarial Parasite Infecting All Blood and Blood-Forming Cells of Birds, J. Infect. Dis. **57**: 315, 1935.
6. Kingsley, D. M.: A Technic for Megakaryocytes and Platelets in Sections, Folia haemat. **57**: 87, 1937.
7. Wright, W. R.: A Note on Mounting Media for Parasitological Materials, Ann. Trop. Med. **21**: 179, 1927.
8. Gilson, G.: Un nouveau médium solidifiable pour le montage des préparations microscopiques, La cellule **23**: 427, 1906.
9. Shepherd, E. S.: A Substitute for Euparal, Tr. Am. Micr. Soc. **37**: 131, 1918.
10. Groat, R. A.: Two New Mounting Media Superior to Canada Balsam and Gum Damar, Anat. Rec. **74**: 1, 1939.
11. Wolbach, S. B.: Studies on Rocky Mountain Spotted Fever, J. M. Research **41**: 1, 1919.
12. Hewitt, R.: A Staining Technic for Demonstrating Avian Malaria Parasites in Tissue Sections, Am. J. Hyg. Sec. C **29**: 115, 1939.
13. Galigher, A. E.: The Essentials of Practical Micro-technique in Animal Biology, Berkeley, 1934, A. E. Galigher, Inc.
14. Kingsley, D. M.: Acetone Solvents for Romanowsky Stains, J. LAB. & CLIN. MED. **22**: 524, 1937.

CHEMICAL

SIMPLIFIED PHOTOELECTRIC PROCEDURES FOR MICRO-DETERMINATION OF NONPROTEIN NITROGEN, UREA NITROGEN, CREATINE, AND CREATININE OF BLOOD*

BARNETT SURE, PH.D., AND VIOLET M. WILDER, PH.D.
FAYETTEVILLE, ARK.

SIMPLE and accurate available microchemical methods for the measurement of the nonprotein nitrogen constituents of the blood of the albino rat all require more blood than can be obtained from the living animal. The spectrophotometer methods of Borsook[1] could not be introduced, since our laboratory did not possess such an instrument. Furthermore, his procedures were considered too involved for routine use. The recently published electrotitration methods of Borsook and Dubnoff,[2] while yielding accurate results, were also considered too complex.

With the aid of the Evelyn Macro-Micro Photoelectric Colorimeter,[3, 4] technique was developed for microdeterminations of nonprotein nitrogen, urea nitrogen, creatine, and creatinine. These procedures are so simple and accurate that they can be carried out by any intelligent hospital technician.

NONPROTEIN NITROGEN

Calibration curves were made using the 520 and 440 filters. The Koch and McMeekin[5] procedure was used for the preparation of the Nessler-Folin reagent for microdeterminations. Instead of working on a 100 ml. basis, the technique adopted was developed on a tenth of a volume basis, i.e., a total dilution to 10 ml., which was carried out in 15 ml. centrifuge tubes graduated in tenths of a milliliter. For the digestion an electrically heated microapparatus, purchased from Eimer and Amend, was used. The Kjeldahl flasks had a total capacity of 10 ml. Tungstic acid blood filtrates were prepared according to the method of Folin,[6] laking the blood with 7 volumes of ammonia-free water, and precipitating the proteins with 1 part of 10 per cent sodium tungstate made up fresh daily, and 1 part of ⅔ normal sulfuric acid.

Procedure.—Measure 0.5 ml. of blood filtrate with a 0.5 ml. delivery pipette into a 10 ml. Kjeldahl flask and add 1 ml. of 1:20 sulfuric acid. Digest until fumes appear and add 1 drop of superoxol. Insert a small funnel against the

*From the Department of Agricultural Chemistry, University of Arkansas, Fayetteville.
Research paper No. 670, Journal Series, University of Arkansas.
Published with the approval of the Director of the Arkansas Agricultural Experiment Station.
Received for publication, May 7, 1940.

fume receiver. Heat again until fumes appear, then heat for one minute, timing with a stop watch. Cool and dilute with about 5 ml. of ammonia-free distilled water. Wash out flask with additional distilled water into a 15 ml. graduated centrifuge tube up to 8.5 ml. mark. Then add 1.5 ml. of the Nessler-Folin reagent.[5] Shake quickly by pouring back and forth from centrifuge tube to absorption tube three times. By such procedure clouding is avoided. Set the blank with reagents at 100 and then read in Evelyn Photoelectric Macro Colorimeter, using the 520 filter, and obtain values from the calibration curve (Fig. 1). The determinations are made in duplicate or triplicate.

The accuracy of the method was tested on two different samples of urine, one on rat's urine and the other on man's urine, using the standard macro-Kjeldahl method for comparison. The results are given in Table I. It is evident from these data that our microprocedure checks to the extent of 1 to 2 per cent with the macro-Kjeldahl method.

TABLE I

URINE	VOLUME (ML.)	NUMBER OF DETERMINATIONS	METHOD	NITROGEN MG. PER ML.	PER CENT ERROR
Rat	0.001	6	Sure and Wilder	58.0	
	1.0	3	Macro-Kjeldahl	58.5	+0.86
	6.0	3	Macro-Kjeldahl	59.0	+1.72
Man	0.02	8	Sure and Wilder	11.52	
	1.0	3	Macro-Kjeldahl	11.76	+2.09

TABLE II

UREA NITROGEN USED FOR ANALYSIS (μG)	UREA NITROGEN FOUND (μG)	PER CENT RECOVERY
20.0	20.00	100.00
20.0	20.05	100.25
30.0	31.00	103.30
30.0	32.00	106.60
40.0	41.00	102.50
40.0	39.00	97.50
40.0	39.00	97.50
50.0	52.00	104.00

UREA NITROGEN

To 0.5 ml. of blood filtrate add 2 drops of a phosphate buffer solution[7] and two drops of a 1 per cent filtered urease solution freshly prepared from Squibb's urease powder. Let stand for one hour at room temperature. Dilute to 7.5 ml. in a 15 ml. centrifuge tube and add 1 ml. of 1:20 sulfuric acid. Transfer from centrifuge tube to absorption tube back and forth twice and shake. This is done to avoid subsequent clouding following addition of the Nessler-Folin reagent. Now add 1.5 ml. of the Nessler-Folin solution. Transfer back and forth to absorption tube three times and shake. Set blank with reagents at 100 and read in photoelectric colorimeter, using the 440 filter, and obtain values from calibration curve (Fig. 1). Recoveries of urea nitrogen by the above micromethod are given in Table II. The nesslerization of the various duplicate or triplicate samples should be done one at a time to prevent clouding.

It is apparent from the results in Table II that our micro urease method yields satisfactory recoveries from known urea solutions. The average error seems to be not greater than ±3.0 per cent.

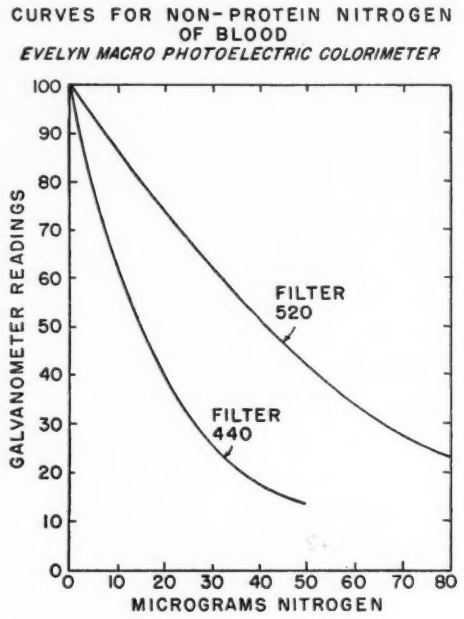


Fig. 1.

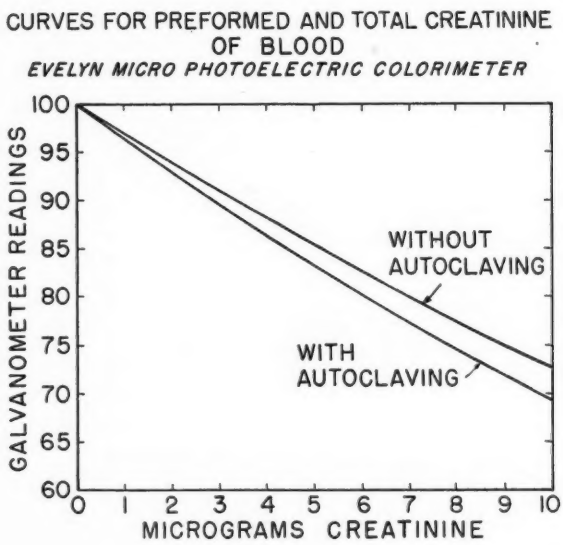


Fig. 2.

PREFORMED CREATININE

The Evelyn Micro Photoelectric Colorimeter was used. The ordinary cadmium-coated plungers corroded with saturated picric acid; therefore, gold-plated plungers made by the Rubicon Company of Philadelphia, manufacturers of the Evelyn Colorimeter, were introduced. The 520-M filter was used.

Procedure.—To 1 ml. of blood filtrate add 0.5 ml. of saturated picric acid solution and let stand for ten minutes. Then add 0.1 ml. of 10 per cent carbonate-free sodium hydroxide. After setting the blank with reagents at 100, read the microcolorimeter and obtain values from the calibration curve (Fig. 2).

All attempts to convert creatine to creatinine with either sulfuric or hydrochloric acid, using the micro cells of the Evelyn Photoelectric Micro Colorimeter, yielded irregular results, and such procedure, therefore, had to be abandoned. The following technique was found very satisfactory: To 1 ml. of blood filtrate add 0.5 ml. of saturated picric acid in a 15 ml. centrifuge tube. Cover with lead foil. Autoclave for forty minutes at 20 pounds pressure. Cool. Add 0.1 ml. of 10 per cent carbonate-free sodium hydroxide and allow to stand for ten minutes. Blank with reagents is also autoclaved for the same time at the same pressure. After setting blank at 100, read in the microcolorimeter and obtain values from a separately calibrated curve (Fig. 2). The plungers should be allowed to be in contact with the picric acid not more than a minute or two and then quickly removed from the cell, rinsed with distilled water, and dried. This is done because of the possibility of corrosion by being allowed too much contact with the saturated picric acid solution, particularly since the gold plating is very thin. The difference between the values for the total creatinine and the preformed creatinine represents the creatine expressed as creatinine.

Recovery of creatinine from creatine hydrate (Eastman Kodak Co.) is given in Table III.

TABLE III

CREATINE HYDRATE USED (μG)	NUMBER OF DETERMINATIONS	THEORETICAL AMOUNT OF CREATININE TO BE RECOVERED (μG)	ACTUAL CREATININE RECOVERED (μG)	PER CENT ERROR
10.0	6	7.51	7.45	−0.79
5.0	7	3.76	3.85	+2.66

It is evident from the above results that the recovery of creatinine from pure creatine by the simplified technique described is almost complete, the experimental error ranging between −0.79 and +2.66.

Total blood creatinine was determined in 16 rats of different ages from our stock colony, the results ranging from 6.7 to 8.65 mg. per 100 ml. of blood. The average was 7.55 mg. per cent.

SUMMARY

Simplified technique is described for the microdeterminations of nonprotein nitrogen, urea nitrogen, creatine, and creatinine in the blood with the aid of the Evelyn Macro-Micro Photoelectric Colorimeter. This technique can be applied to capillary blood of small laboratory animals as well as human beings.

REFERENCES

1. Borsook, H.: Micromethods for Determination of Ammonia, Urea, Total Nitrogen, Uric Acid, Creatinine (and Creatine), and Allantoin, J. Biol. Chem. **110**: 421, 1935.
2. Borsook, H., and Dubnoff, J. W.: Methods for the Determination of Submicro Quantities of Total Nitrogen, Ammonia, Amino Nitrogen, Amides, Peptides, Adenylic Acid, and Nitrates, J. Biol. Chem. **131**: 163, 1939.

3. Evelyn, K. A.: Stabilized Photoelectric Colorimeter With Light Filters, J. Biol. Chem. 115: 63, 1936.
4. Evelyn, K. A.: Photoelectric Microcolorimeter, Ibid. 117: 365, 1937.
5. Koch, F. C., and McMeekin, T. L.: A New Direct Nesslerization Micro-Kjeldahl Method and a Modification of the Nessler-Folin Reagent for Ammonia, J. Am. Chem. Soc. 46: 2066, 1924.
6. Folin, O.: Laboratory Manual of Biological Chemistry, New York, 1926, D. Appleton-Century Company, p. 227.
7. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, 1932, Williams & Wilkins Co., Vol. II, Methods, p. 547.

CLINICAL APPLICATION OF THE MICRODIFFUSION METHOD FOR THE ESTIMATION OF ACETONE*

S. C. WERCH, M.Sc., M.D., CHICAGO, ILL.

IN A RECENT publication[1] regarding the microdiffusion method for the estimation of acetone, the theory and work on which the method is based was discussed. Reference was also made to the clinical application of the method. The purpose of this paper is to offer a rapid procedure which may be used in a clinical laboratory. A comparison is also made between results obtained by this method and by the colorimeter method of Behre and Benedict.[2]

APPARATUS AND TECHNIQUE

The microdiffusion apparatus is the Conway unit. This unit consists of a small dish of fairly thick glass, resembling a Petri dish (Fig. 1).[3] It is divided into two chambers by a circular wall of glass arising from the floor of the dish to about half the height of the outer wall. When in use it is covered with a transparent glass plate and sealed with a fixative.

The Deniges reaction[4] of acetone precipitation as a basic mercuric compound is utilized. The factors concerned are the time necessary to cause precipitation and the temperature at which the reaction takes place. Two cubic centimeters of Nessler's solution, three cubic centimeters of unknown, and one cubic centimeter of 1 per cent hydrochloric acid are required. Since acetone is volatile, the unknown (urine or oxalated whole blood) should be stoppered as soon as collected and used fresh. The acid is used to fix any ammonia which may be present in the unknown.

Carefully cleaned units are obligatory. This may be accomplished by dipping into cleaning solution and then rinsing consecutively with water, alcohol, and ether.

When ready for use, the units with their covers are placed on a black background, and the edges of the outer wall are smeared with vaseline. Next, the reagents are introduced. First, the central chamber receives the Nessler's solution, and then the acid is run in, distributing it evenly around the outer chamber. The transparent glass plate is next placed to cover the entire unit, except for a small slit, permitting the introduction of the end of the pipette used to carry the unknown—urine or oxalated whole blood. A stop watch held in one hand is

*From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.
Received for publication, February 10, 1940.

Fig. 1.

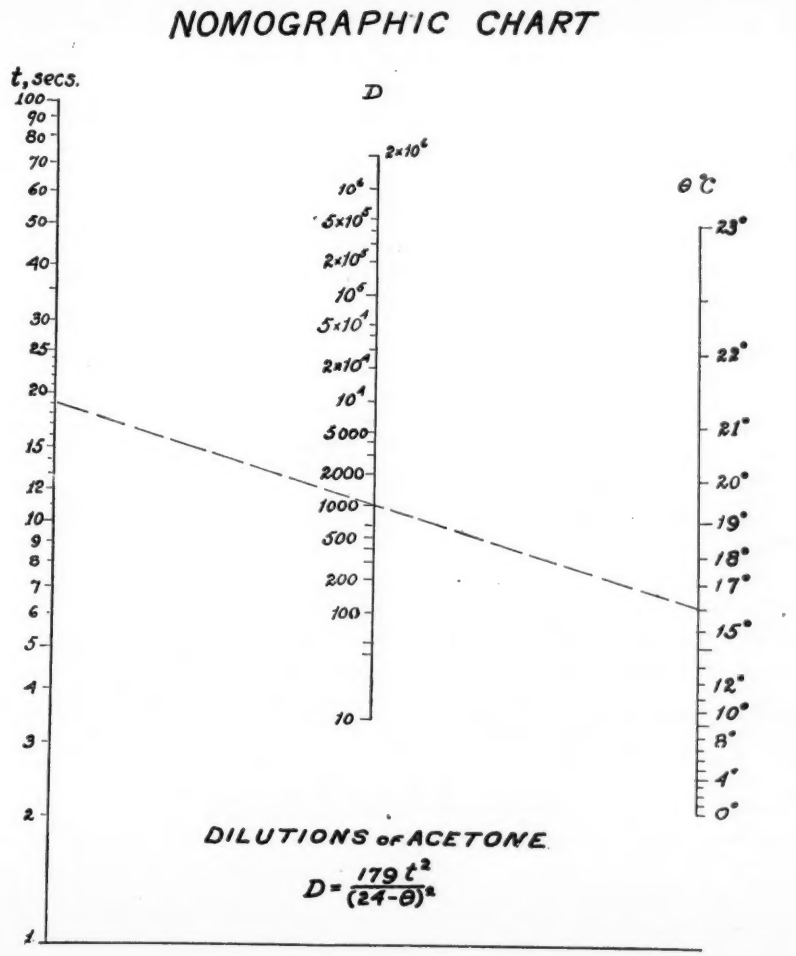

NOMOGRAPHIC CHART
t, secs.
D
θ°C
100
90
80
70
60
50
40
30
25
20
15
12
10
9
8
7
6
5
4
3
2
1
2×10⁶
10⁶
5×10⁵
2×10⁵
10⁵
5×10⁴
2×10⁴
10⁴
5000
2000
1000
500
200
100
10
23°
22°
21°
20°
19°
18°
17°
15°
12°
10°
8°
4°
0°
DILUTIONS of ACETONE
D = 179 t² / (24-θ)²

Fig. 2.

started as soon as the pipette containing the unknown, held by the other hand, stops flowing freely. The hand holding the watch next slides the glass plate so that it completely covers the unit, as an immediate gesture after starting the watch. When the endpoint is reached the watch is stopped. Rapid introduction of the unknown is best done with a pipette possessing a large opening. The endpoint is the appearance of precipitation of the Nessler's solution at the periphery of the inner chamber of the unit in the form of threads, like spokes of a wheel, or as a cloud. It is that second when the spokes or cloud extend inward 1 mm.

The temperature factor may be controlled without great loss of accuracy by placing the reagents and utensils in a cool (20° C.) corner of the laboratory for an hour. The reading on a thermometer hung close by may be used. Time readings varying no more than ±2 seconds from three tests may be averaged and considered sufficient. The temperature and time factors thus obtained are then located on the nomogram (Fig. 2) with a straight edge, and the concentration of acetone is read off in the center.

COMPARISON WITH BEHRE-BENEDICT METHOD

It was considered well to compare this method with another of proved value.

The following dilutions of specified acetone in human urine and oxalated ox blood were made from a 1 per cent solution and estimated by the two methods: 1:500, 1:1,000, 1:3,000, 1:7,000, and 1:10,000. Incubation of the apparatus and reagents was again resorted to in our method in order to control better the temperature factor. Table I shows the values obtained.

TABLE I

ACETONE IN MILLIGRAMS PER 100 C.C. OF URINE OR BLOOD

DILUTIONS	ACETONE IN HUMAN URINE		ACETONE IN OXALATED WHOLE OX BLOOD	
	MICRODIFFUSION METHOD	BEHRE-BENEDICT METHOD	MICRODIFFUSION METHOD	BEHRE-BENEDICT METHOD
1:100	95.00	98.00	90.00	95.00
1:500	19.50	19.50	19.00	19.50
1:1,000	10.00	9.50	11.00	9.50
1:3,000	3.50	3.00	3.00	3.50
1:7,000	1.50	2.00	1.50	2.50
1:10,000	1.20	1.50	1.30	1.80

SUMMARY

The microdiffusion method for the estimation of acetone has been modified to fit the needs of the clinical laboratory. With a little practice a urine or blood sample may be determined in five to ten minutes.

This work was begun at Trinity College, Dublin, under the direction of Professor W. R. Fearon, of the Department of Biochemistry. I wish to thank him for his interest and kind assistance. I also wish to thank Mr. Einhart Kameran, who took part in the development of the technique.

REFERENCES

1. Werch, S. C.: A Microdiffusion Method for the Estimation of Acetone, J. LAB. & CLIN. MED. **25**: 414, 1940.
2. Behre, J. A., and Benedict, S. R.: Colorimetric Method for the Estimation of Acetone, J. Biol. Chem. **70**: 487, 1926.
3. Gallenkamp, A., and Co., Ltd., 17-29 Sun Street, Finsbury Square, London, E. C. 2.
4. Deniges, F.: Précis de Chimie Analytique, ed. 6, Lyon, 1930, A. Stork & Cie, p. 213.

A CLINICAL METHOD FOR EXTRACTION OF URINARY ANDROGENS PRELIMINARY TO COLORIMETRIC QUANTITATION BY OESTING'S TECHNIQUE*

W. Kenneth Cuyler,† M.A., and Margaret Baptist
Durham, N. C.

ALL those engaged in the quantitation of urinary androgens have felt the need of a method less expensive and requiring a shorter period of time for completion than those of bio-assays which employ capons or baby chicks. It was with enthusiasm, therefore, that clinicians and research workers turned to the reports of Zimmermann for a chemical method for the determination of urinary androgens.

Zimmermann demonstrated[1, 2] that the steroid sex hormones could be determined quantitatively by the use of meta-dinitrobenzene by means of a characteristic color reaction which occurred in the presence of an alkali with compounds containing an active methylene group. Since his work was done chiefly with crystalline sex steroids, an application of the "Zimmermann reaction" had to be devised for urinary extracts.

Several such methods had been described as suitable for the determination of urinary androgens when the procedure described herein was initiated.[3-5] Careful consideration was given to these methods, with particular regard to the expense of new equipment necessary, the relative simplicity of the technique, the time elements involved, and the practicality of doing consecutive daily assays on a number of patients. Oesting's method[4] seemed to satisfy best our requirements.

The method for extraction used by Oesting is essentially that of Gallagher and Koch.[6] In their procedure benzol was the solvent employed and the extraction was carried out in a continuous extractor. We have modified the method of extraction so as to eliminate the use of benzol and the continuous extractor. Although the latter is very efficient, it is of such dimensions as to be impracticable in the smaller clinical laboratories. Because of its high toxicity and inflammability, benzol is a dangerous solvent to use in the average laboratory space.

The extracting medium employed in the procedure described below is dibutyl ether.‡ Besides being a thoroughly satisfactory solvent[7, 8] for steroids, dibutyl ether has a number of advantages over other solvents. It is practically nontoxic and is nonirritating to the mucosa of the air passages. It has a low kindling

*From the Endocrine Division of the Department of Obstetrics and Gynecology, Duke University School of Medicine and Hospital, Durham.
Received for publication, February 19, 1940.
†Ciba Fellow in Clinical Endocrinology.
‡Secured from the Union Carbide and Carbon Corporation, Long Island City, New York.

point, and, a fact which is very important, urinary pigments are only slightly soluble in it. The extractions can be made in a simple apparatus. The recovery after distillation is approximately 90 per cent, and the solvent can be used a second time before redistillation is necessary.

METHOD OF EXTRACTION

An aliquot fraction of a twenty-four-hour specimen of urine (not less than 200 c.c., if possible) is acidified, using 5 c.c. of concentrated sulfuric acid (reagent) per 100 c.c. of urine. Hydrolysis is accomplished by autoclaving for fifteen minutes at 15 pounds pressure. When cool the urine is extracted three times with dibutyl ether. Fresh solvent is used for each extraction. For specimens which are more than 500 c.c. in volume, 20 c.c. of solvent per 100 c.c. are used; for specimens of less than 500 c.c. in volume, only two extractions are made, using 150 c.c. of solvent for each extraction. The extraction is carried out in thin-walled, globe-shaped 2L separatory funnels by violent shaking by hand at the rate of 180 shakes per minute for three minutes for each extraction.

The combined dibutyl ether extracts are washed with (1) 100 c.c. of 10 per cent sodium hydroxide, (2) 100 c.c. of sodium bicarbonate solution saturated at room temperature, and (3) 100 c.c. of tap water. These amounts are used when the amount of dibutyl ether extract is 300 c.c. or less. Proportionately more of the solutions must be used if the volume of dibutyl ether extract is greater than 300 c.c. (By washing the dibutyl ether extract with the two alkalies, the estrogens are partially removed. These are completely removed in a subsequent step. The strong alkali breaks down the emulsion which sometimes had formed and removes most of the pigment which had been extracted by the solvent.)

The washed dibutyl ether extract is then transferred to a 2L distillation flask, and the solvent is removed by steam distillation under reduced pressure. When dry the flask is washed out three times with ethyl ether, using a total of 100 c.c. (Anesthetic ether is used in order to employ a product as nearly peroxide and aldehyde free as possible.)

The ethyl ether extract of the dry residue is transferred to a 125 c.c. separatory funnel where it is washed with 20 c.c. portions of 10 per cent sodium hydroxide until the washings are clear. This usually requires three to five washings, occasionally more. The ethyl ether extract is then washed three times with 20 c.c. of distilled water.

The ethyl ether extract is transferred to a 250 c.c. beaker containing approximately 0.5 Gm. of decolorizing charcoal.* The solution is thoroughly stirred, and the charcoal is allowed to settle out before filtering. The charcoal is well washed with additional ether. To complete the final step in the extraction of the urinary androgens, the ether is allowed to evaporate or it is evaporated to dryness over a steam bath.

Occasionally faulty filter paper will allow the passage of some charcoal. This cannot be observed until the ether is all evaporated. In this event the extract should be taken up in 100 c.c. of ether and refiltered. Although discoloration due to charcoal is obvious, there occasionally appears a brownish residue in the beaker after evaporation of the ether, for

*"Norit-A," purchased from the Arthur H. Thomas Co., Philadelphia, Pa.

which no explanation is offered. This brown residue is difficult to remove by further treatment with charcoal. Moreover, additional treatment with charcoal and subsequent filtering so greatly reduces the steroid content of the ether extract that subsequent colorimetric determinations are valueless.

New lots of charcoal should be checked against the charcoal being used with regard to steroid adsorption and general quality of the powder. One brand of charcoal allowed no measurable amount of steroids to pass through the filter. Other brands have given trouble with regard to colored or muddy-appearing residues after evaporation of the ether extract.

COLORIMETRIC DETERMINATION

No changes have been made in the colorimetric determination part of Oesting's procedure. The colorimeter used is one designed by Oesting with the assistance of the Hellige Manufacturing Co.

It has been our constant practice that one of us (W.K.C.) attend to the extraction of specimens and the other (M.B.) carry out the colorimetric procedure. We believe that in this manner personal error has been minimized.

The following data, representing 220 colorimeter readings, illustrate the accuracy which is obtained by careful technique:

110 determinations made in duplicate
68 of these, or 62 per cent, matched
42 of these, or 38 per cent, did not match

Of the 42 which did not match
15 differed by only 0.1 color unit
9 differed by only 0.2 color unit
7 differed by only 0.3 color unit
8 differed by only 0.4 color unit
2 differed by only 0.5 color unit
1 differed by only 0.7 color unit

Although this method does not permit qualitative identification of any member of the androgen group, it is felt that these data on total "androgens" as a group (17-ketosteroids) are of very definite value. This value is enhanced by consecutive daily determinations. It has been shown by our group that there is a marked variation in the daily excretion of these compounds by women.[9] These variations take on some significance in view of the apparent accuracy of the method.

SUMMARY

A clinical method for extraction of urinary androgens, preliminary to colorimetric quantitation, has been described. Dibutyl ether is substituted for benzol as the solvent. The advantages of dibutyl ether are enumerated.

The method of extraction is adaptable to the average laboratory and is practicable for assaying consecutive daily specimens on a number of patients concurrently.

We wish to express our indebtedness and thanks to Dr. E. C. Hamblen, Chief of Endocrine Division, for his constant support and helpful criticism.

REFERENCES

1. Zimmermann, W.: Eine Farbreaktion der Sexualhormone und ihre Anwendung zur quantitativen colorimetrischen Bestimmung, Ztschr. f. physiol. Chem. **233**: 257, 1935.
2. Zimmermann, W.: Colorimetrische Bestimmung der Keimdrüsenhormone, Ztschr. f. physiol. Chem. **245**: 47, 1936.

3. Wu, H., and Chou, C.-Y.: Colorimetric Methods for the Determination of Sex Hormones in Human Urine, Chinese J. Physiol. **11**: 413, 1937.
4. Oesting, R. B.: Colorimetric Assay for Male Sex Hormones in Urine, Proc. Soc. Exper. Biol. & Med. **36**: 524, 1937.
Oesting, R. B.: Colorimetric Assay of Urinary Androgens, Proc. Soc. Exper. Biol. & Med. **39**: 76, 1938.
5. Callow, R. K., and Emmens, C. W.: Colorimetric Determination of Substances Containing Grouping—CH_2CO—in Urine Extracts as Indication of Androgen Content, Biochem. J. **32**: 1312, 1938.
6. Gallagher, T. F., and Koch, F. C.: Quantitative Assay for Testicular Hormone by Comb-Growth Reaction, J. Pharmacol. & Exper. Therap. **55**: 97, 1935.
7. McCullagh, D. R., and McLin, T. R.: Extraction of Androgens From Urine, Endocrinology **22**: 120, 1938.
8. McCullagh, D. R., and Guillet, R.: Summary of Some Laboratory Methods in Endocrinology, Cleveland Clin. Quart. **6**: 95, 1939.
9. Hamblen, E. C., Ross, R. A., Cuyler, W. K., Baptist, M., and Ashley, C.: Studies on the Metabolism of Androgens in Women, Endocrinology **25**: 491, 1939.

SYRINGE TYPE GLASS ELECTRODE FOR THE MEASUREMENT OF BLOOD pH*

OTTO H. MÜLLER, SC.D., AND E. COOPER PERSON, JR., M.D.
NEW YORK, N. Y.

MANY glass electrodes have been designed for measuring the pH of blood as it exists in the body, but there are relatively few that are free from objections. To be satisfactory for blood analysis, the electrode not only must conform to the usual standards for glass electrodes but also must meet the following additional requirements:

(1) Analysis of small samples of blood should be possible.
(2) The blood must not be altered by coming into contact with air or by getting mixed with another solution.
(3) The blood should not undergo marked changes in temperature.
(4) The pH of the blood must be measured quickly, before glycolysis sets in.
(5) The blood should be obtained without stasis and without the use of suction by means of a syringe.

In our opinion, the electrode described here† meets all these requirements and has additional features which make it suitable for other studies. We have used it for over a year with very satisfactory results.

The drawing in Fig. 1 shows the construction of the electrode. It is a modification of the type developed by MacInnes and Belcher[1] in which the glass membrane is well protected because it forms the inner tube of the electrode. The tip at *A* is ground to fit a standard Luer hypodermic needle. The ground surface at *B* makes it possible to seal the electrode with a glass stopper, or to

*From the Department of Surgery of the New York Hospital and Cornell University Medical College.
Supported by a grant from the John and Mary B. Markle Foundation.
Received for publication, February 23, 1940.
†Obtainable from Mr. Otto Hopf, Flushing, N. Y.

introduce the tip of a standard syringe so that the electrode forms an extension of the barrel of the syringe. The over-all length of the electrode is 9 cm., and only 0.5 c.c. of blood is necessary to fill it completely. At *C* a reference solution and electrode are introduced; we have used 0.1 N hydrochloric acid and a silver-silver chloride electrode,[2] sealed into place with De Khotinsky cement. The electrode is rugged and convenient to handle; furthermore, the straight glass membrane can be easily cleaned by means of a soft feather and water.

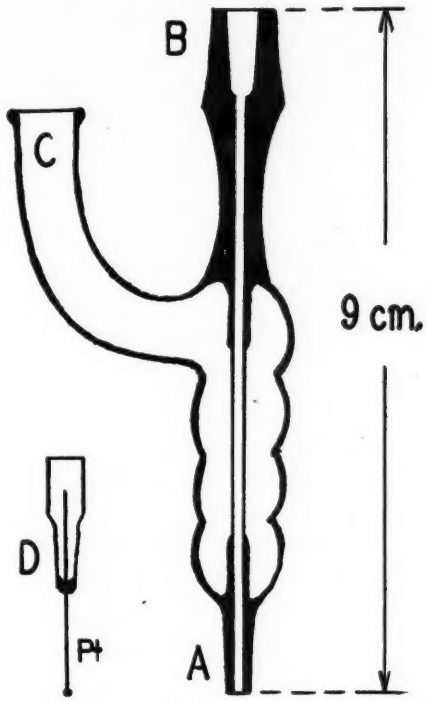


Fig. 1.—Syringe type glass electrode and special plug for measurement of oxidation-reduction potentials.

The measurements were made with a Beckman pH meter which permitted readings accurate to 0.03 pH units. To insure constant temperature the glass electrode and calomel half cell are hung in a saturated solution of potassium chloride which is kept at the desired temperature by circulating water. A sketch of the small water bath which was constructed to fit the Beckman pH meter is shown in Fig. 2. A simple brass clip attached to a bakelite block (Fig. 2) insulates the glass electrode from the pH meter and holds it in place. The potassium chloride solution serves further as a liquid junction between the glass electrode and the calomel half cell.

Often it is desirable to measure oxidation-reduction potentials as well as the pH of the same solution. If instead of the glass stopper, a special plug (Fig. 1, *D*) is used into which a platinum wire has been sealed, both measurements can be carried out on the same sample and with the same meter.

Operation of the Electrode.—The electrode is warmed to the desired temperature and calibrated with several buffers of known pH. It is advisable to use buffers with pH values near that of the unknown. When repeated meas-

urements prove the satisfactory performance of the electrode, it is washed in the warm circulating water and rinsed with a warm solution of heparin in physiologic saline solution. A sterile hypodermic needle is then attached to tip *A* and the blood vessel is punctured. During the sampling the electrode temperature is kept near body temperature by holding it covered with the hand. If arterial blood is taken, the electrode will fill very quickly because little resistance is offered by the straight barrel of the electrode to the flow of blood.

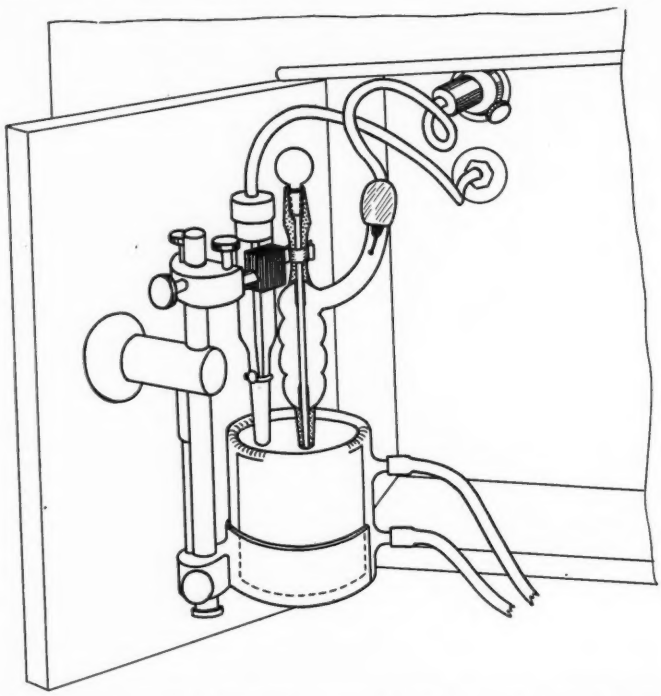


Fig. 2.—Sketch showing glass electrode, calomel half cell, and water bath attached to door of pH meter. Raising the water bath will immerse the electrodes in a saturated solution of potassium chloride which serves as liquid junction.

If venous blood is taken, the electrode has to be held in a horizontal position, or slight stasis of the vein becomes necessary to force the blood through the electrode. In addition to the 0.5 c.c. which is necessary to fill the electrode, another 0.5 c.c. of blood is passed through the electrode. This washes out any excess heparin so that the composition of the blood should be the same as that in the blood vessel. While the blood is still flowing, the electrode is stoppered. It is then withdrawn and hung into the potassium chloride solution after the needle has been removed. Stable potentials are obtained immediately. The whole procedure requires less than one minute.

The pH values obtained are reproducible if the blood samples are taken from the same person within an hour. In agreement with the observations of others,[3, 4] we found that the presence of heparin does not have a measurable effect on the pH; neither does clotting alter the observed pH[5]. However, if the blood is left in the electrode more than five minutes, a drift to a lower pH, due to glycolysis, is noticeable.[4, 5] Some studies made with this electrode on the blood pH of normal and hypertensive dogs are published elsewhere.[6]

REFERENCES

1. MacInnes, D. A., and Belcher, D.: A Durable Glass Electrode, Indust. & Engin. Chem. Anal. Ed. **5**: 199, 1933.
2. Brown, A. S.: A Type of Silver Chloride Electrode Suitable for Use in Dilute Solutions, J. Am. Chem. Soc. **56**: 646, 1934.
3. Yoshimura, H.: A New Micro-Glass Electrode and the pH of Arterial, Venous and Capillary Blood, J. Biochem. **23**: 335, 1936.
4. Haugaard, G., and Lundsteen, E.: Über die Messung des pH im Blute mit Hilfe der Glaselektrode, Biochem. Ztschr. **285**: 270, 1936.
5. Ferguson, J. H., and DuBois, D.: Observations on the pH of Clotting and Citrated Blood, J. Lab. & Clin. Med. **21**: 663, 1936.
6. Müller, O. H., and Nickel, W. F.: The pH of Systemic Blood in Normal and Hypertensive Dogs, Determined by Means of a Syringe Type Glass Electrode, Proc. Soc. Exper. Biol. & Med. **43**: 89, 1940.

COMPARATIVE STUDY ON A NEW SIMPLE METHOD OF SULFANILAMIDE DETERMINATION IN BLOOD*

Mason C. Andrews, B.A., and Arnold F. Strauss, M.D.
Norfolk, Va.

THE extensive use of sulfanilamide treatment and the recognized importance of attaining and maintaining an optimal blood concentration make it very desirable to have a method which combines simplicity and accuracy in estimating this blood concentration.

In this country the method developed by Marshall (1937) is accepted as satisfactory. Recently, Werner (1939) described in *Lancet* a novel method for which he claims advantages over Marshall's diazotization reaction; namely, simplicity of technique, which results in a considerable saving of time, and the use of only reliable reagents, since the new method eliminates the use of the unstable coupling agent, dimethylnaphthylamine. This method is based on the direct formation of a yellow color when Ehrlich's reagent, para-dimethylaminobenzaldehyde, is added to the filtrate from blood containing sulfanilamide.

There has been no report of investigation of this method in American literature. The purpose of this publication is to compare Werner's method with that of Marshall.

About fifty determinations of free sulfanilamide in blood in varying cases have been made by this method. The following procedure incorporating some modifications was found satisfactory: 2 c.c. of oxalated blood are added drop by drop from a pipette to 8 c.c. of a 5 per cent solution of trichloracetic acid. The blood protein is thereby immediately precipitated and is easily filtered off, yielding a clear filtrate. Next 3 c.c. of fourth-normal sodium hydroxide is added to 4 c.c. of filtrate, followed by 1 c.c. of Ehrlich's reagent. The yellow color develops immediately to its maximal intensity. It is compared in the colorimeter to the color developed by adding 1 c.c. of the reagent to 7 c.c. of a standard solution of sulfanilamide in distilled water, or by diluting 1.25 c.c. of the reagent to 10 c.c. with the standard. The standard, containing 0.08 mg. of sulfanilamide

*From the Pathological Laboratory of St. Vincent's Hospital, Norfolk.
Received for publication, February 24, 1940.

per 7 c.c. of water, is conveniently prepared by dissolving 0.8 Gm. of sulfanilamide in 700 c.c. of water, or 1.143 Gm. of sulfanilamide in 1,000 c.c. of water and diluting 1:100.

If the unknown is set at 10 in the colorimeter, the readings of the standard will be milligrams per hundred cubic centimeter of blood. When the concentration of the unknown exceeded 10 mg., exact results were not obtained. However, if the unknown is set at 5 and the readings of the standard are multiplied by 2, the results are exactly accurate.

The reagents used include: 5 per cent trichloracetic acid, Ehrlich's reagent (3 Gm. of para-dimethylaminobenzaldehyde added to 100 c.c. water containing 3 c.c. of concentrated sulfuric acid), and fourth-normal sodium hydroxide.

TABLE I

MG. SULFANILAMIDE IN 100 C.C. BLOOD	AVERAGE RESULTS BY METHOD OF:	
	WERNER	MARSHALL
2.5	3.1	2.5
5.0	5.1	5.0
7.2	7.0	6.8
9.0	8.9	8.9
10.3	10.6	10.1
13.0	13.0	12.9
15.0	14.9	14.8

Table I shows the results obtained by Marshall's method compared with those obtained by Werner's method. Examination of blood to which sulfanilamide has been added to produce known concentrations showed essentially identical results.

Examination of the blood of sulfanilamide-treated patients likewise yielded the same results by both methods. There has not been any interference by other medications given to the patient.

Werner's method is accomplished by an extremely simple procedure which seems to make it superior for large scale use and saves considerable time for a single determination. The time required for one test by Marshall's method was about fifty minutes, although only fifteen minutes of that time was spent in actual manipulation. A determination by Werner's method can be completed in twelve minutes. The stability of the color developed by this procedure makes possible the use of a single standard solution for a large number of determinations. It fails to show appreciable change after two weeks' time. Ample time is available for reading the tests, and a number may be done simultaneously and read when convenient. The color formed by Marshall's procedure deteriorates rapidly, and each test must be read at a definite time, eliminating the possibility of carrying out many tests entirely simultaneously. A new standard must be made at least for each series of determinations.

Although the dimethylaminobenzaldehyde solution has a slight yellowish color, the distortion caused by it is not significant, except at very low concentrations (Table I). If the dimethylaminobenzaldehyde crystals are very old and show a greenish-brown color, the resulting solution is too yellow for use.

Werner's method is equally satisfactory for sulfapyridine determination. A sulfapyridine standard may be made or the sulfanilamide standard used and the result multiplied by 1.5 to correct for differences in molecular weight.

Werner also describes a very simple procedure for the determination of sulfanilamide in urine. He further gives directions for making a permanent set of standards from potassium chromate for use in a color comparator. Their use did not prove to be very satisfactory.

A disadvantage of Werner's method is that the yellow color developed is more difficult to compare in the colorimeter than the reddish one obtained by Marshall's procedure.

SUMMARY

A new method for determination of sulfanilamide in blood developed by Werner has been compared with that of Marshall. This method gives accurate results while allowing a considerable saving of time. The reagents used, as well as the standard solution, are stable.

The disadvantage of this method is its use of yellow for colorimetry.

REFERENCES

Marshall, E. K., Jr.: Determination of Sulfanilamide in Blood and Urine, J. Biol. Chem. **122**: 263, 1937.

Marshall, E. K., Jr., Cutting, W. C., and Emerson, K., Jr.: Acetylation of Para-Aminobenzenesulfonamide in Animal Organism, Science **85**: 202, 1937.

Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C.: Para-Aminobenzenesulfonamide; Absorption and Excretion; Methods of Determination in Urine and Blood, J. A. M. A. **108**: 953, 1937.

Werner, A. E. A.: Estimation of Sulfanilamide in Biological Fluids, Lancet **1**: 18, 1939.

RAPID VOLUMETRIC DETERMINATION OF URINARY SODIUM*

EVAN W. MCCHESNEY, PH.D., RENSSELAER, N. Y.

RECENTLY Strauss[1] demonstrated that urine, to which has been added thorium nitrate and sulfuric acid, can be ashed very rapidly in silica beakers in an electric muffle furnace. The method was developed primarily for the determination of potassium and, although the author suggested that it might be adapted to the determination of sodium, she did not establish the fact that this could be done. Her method is much more convenient than the older one of Stolte[2] as applied by Tisdall and Kramer[3] which is both tedious and time-consuming.

We wished, in this laboratory, to make a large number of sodium determinations and none of the methods now available seemed suited to our needs because of the time they required. We, therefore, decided to try to combine the rapid ashing technique of Strauss with the determination of sodium as the pyroantimonate by the method of Kramer and Gittleman,[4] and we found that such a combination was very satisfactory. With these combined techniques one may determine sodium in duplicate on as many as eight urine samples in about five hours of elapsed time, or about three hours of actual work.

*From the Research Laboratories, Winthrop Chemical Co.
Received for publication, April 15, 1940.

Reagents.—All solutions are prepared as described in the original articles with one exception: the solution used to extract the ash must be 0.4 per cent phosphoric acid instead of the 0.1 per cent solution recommended by Strauss. The reasons for this will be set forth below. This reagent, 10 per cent thorium nitrate, 10 per cent potassium hydroxide, and potassium pyroantimonate reagent are kept in paraffin-lined bottles.

Procedure.—An amount of urine containing 15 to 25 mg. of sodium (ordinarily this would be 5 to 7 c.c.) is measured into a silica beaker of 50 or 100 c.c. capacity, together with 1 c.c. of 10 per cent thorium nitrate and 1 c.c. of 4 N sulfuric acid. The material is evaporated on the water bath, ashed, and extracted (with 0.4 per cent phosphoric acid), as described by Strauss. Samples of 1 c.c. of the extract are taken for analysis. To these samples are added 4 drops of 10 per cent potassium hydroxide to neutralize the phosphoric acid, after which the procedure followed is that of Kramer and Gittleman as modified by Eisenman.[5]

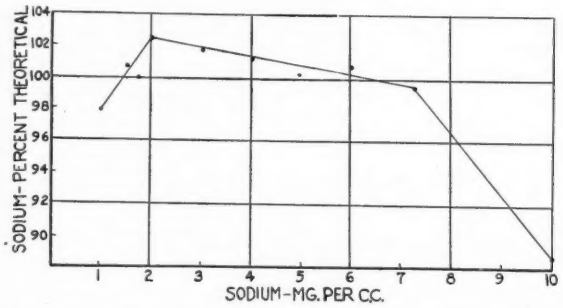


Fig. 1.—Relationship of concentration of sodium to the amount found.

Interference of Thorium.—In her article Strauss states that the ashes extracted with 0.1 per cent phosphoric acid are thorium free, but this has not been our experience. Such extracts contain considerable amounts of thorium, which is precipitated by the pyroantimonate reagent, and therefore give high results. Data bearing on this point are given in Table I.

TABLE I

INTERFERENCE OF THORIUM WITH SODIUM DETERMINATION

SAMPLE NUMBER	H_3PO_4 USED FOR EXTRACTION (%)	SODIUM TAKEN* (MG.)	SODIUM FOUND (MG.)	BLANK VALUE
1	0.1	3.14	3.83	0.69
2	0.1	2.61	3.31	0.70
3	0.1	0.00	0.70	0.70
4	0.4	3.14	3.27	0.13
5	0.4	0.00	0.17	0.17

*Per cubic centimeter of ash solution.

Limits of the Method.—The work of Kramer and Gittleman indicated their method to be accurate within ± 2 per cent. Balint[6] has claimed that the method gives results which are about 3 per cent higher than the theoretical, and Peters and Van Slyke[7] suggest that the results be divided by a factor of 1.03. It should be noted that Eisenman, and Peters and Van Slyke, give the impression that the method is accurate only when the amount of sodium determined

is between 2 and 3.75 mg. per cubic centimeter of ash solution. Balint, on the other hand, gives the permissible range as 0.5 to 6 mg. We felt that this point was worthy of further study and have found that the range of 1.0 to 7.5 mg. gives a reasonable degree of accuracy (Fig. 1).

TABLE II

ANALYSIS OF URINE WITH ADDED SODIUM CHLORIDE

SAMPLE NUMBER	COMPOSITION OF SAMPLE (C.C.)		SODIUM FOUND TOTAL (MG.)	SODIUM ADDED (MG.)	SODIUM RECOVERED (MG.)	PER CENT RECOVERY
	URINE	0.8% NaCl				
1	5	0	18.80	0		
2	5	1	21.97	3.14	3.17	100.9
3	4	2	21.39	6.29	6.35	100.9
4	3	3	20.82	9.43	9.54	101.2
5	2	4	20.14	12.58	12.62	100.3
6	1	5	18.51	15.72	14.75	93.8*

*The results have invariably been low when 1 c.c. samples of urine (and, occasionally, 2 c.c. samples) were ashed, probably because of the bulky and gelatinous precipitates formed in the ash solutions. Apparently in these cases a smaller proportion of the thorium is converted to the oxide, hence more is left to form the phosphate. A similar situation was encountered in analyzing the synthetic urines described in Table III; this probably explains the low results obtained on these samples.

TABLE III

ANALYSIS OF MIXTURES OF KNOWN COMPOSITION

SUBSTANCE	SAMPLE A	SAMPLE B	SAMPLE C
NaCl	20 Gm.	10 Gm.	None
Other salts*	10.25 Gm.	10.25 Gm.	10.25 Gm.
Urea	20 Gm.	20 Gm.	20 Gm.
Water added	1,200 c.c.	1,200 c.c.	1,200 c.c.
Mg. sodium per c.c., theory	6.55	3.27	0.0
Mg. sodium per c.c., found	6.31	3.20	0.0

*The composition of this mixture was 6 Gm. of KH_2PO_4, 1.1 Gm. of $CaCl_2.2H_2O$, 0.65 Gm. of $MgSO_4$, and 2.5 Gm. of $(NH_4)_2SO_4$.

Accuracy of the Method as Applied to Urine.—This accuracy has been demonstrated in three ways: (1) recovery of sodium added to urine (Table II), (2) analysis of known mixtures approximating the composition of urine (Table III), and (3) demonstration of the agreement of the values obtained with those of the gravimetric method of Butler and Tuthill.[8] It might be stated further that the method is remarkably precise, since it is not at all unusual to have all the pairs of five duplicate determinations agree within 0.02 mg. sodium (that is, about 0.7 per cent difference), and differences of as much as 0.05 mg. are very rare.

REFERENCES

1. Strauss, M. B.: The Use of Thorium Nitrate in the Rapid Ashing of Serum and Urine, J. Biol. Chem. **118**: 331, 1937.
2. Stolte, K.: Eine einfache und zuverlässige Methodik der Aschenanalyse, Biochem. Ztschr. **35**: 104, 1911.
3. Tisdall, F. F., and Kramer, B.: Methods for the Direct Quantitative Determination of Sodium, Potassium, Calcium, and Magnesium in Urine and Stools, J. Biol. Chem. **48**: 1, 1921.
4. Kramer, B., and Gittleman, I.: An Iodometric Method for the Determination of Sodium in Small Amounts of Serum, Ibid. **62**: 353, 1924.
5. Wakeman, A. M., Eisenman, A. J., and Peters, J. P.: A Study of Human Red Blood Cell Permeability, Ibid. **73**: 567, 1927.
6. Balint, M.: Eine jodometrische Mikrobestimmung des Natriums, Biochem. Ztschr. **150**: 425, 1924.
7. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Methods, Baltimore, 1932, Williams & Wilkins Co., pp. 737-740.

THE STANDARDIZATION OF pH MEASUREMENTS WITH A PRECISION GLASS ELECTRODE AT VARIOUS TEMPERATURES*

ALBERT GLAUBIGER, M.S., NEW YORK, N. Y.

INTRODUCTION

THE pH values of a number of biological products and bacterial media, obtained by colorimetric and electrometric methods, have been presented in some recent papers.[1, 2] In several procedures employed, the test fluids were diluted with fixed quantities of distilled water or physiologic saline solution. In the present work the pH values of a number of buffer mixtures, diluted with varying quantities of distilled water and saline solution, have been determined between the temperatures of 10° C. and 38° C. Since the measurement of pH values is so fundamental in all biological laboratories, the use of a standard pH scale is essential. The pH scale adopted throughout this work is based upon the pH values of primary potassium phthalate. The pH values of the buffer mixtures studied here have been tested by employing these values in computing the dissociation constants of the corresponding buffer acids by means of an approximation of the Debye-Hückel limiting law.

Earlier work on the standardization of the pH scale has been fully described by Clark.[3] Recent studies of Hitchcock,[4, 5] MacInnes,[6] and their associates have provided a fundamental basis for further pH studies. Our work is based essentially upon the methods employed by these workers.

The dissociation constant K′ of a weak acid HA is a function of the pH value of a solution of the acid and its salt:

$$pK' = pH - \log \frac{C_A}{C_{HA}} \quad (1)$$

By replacing the stoichiometric concentrations C_A and C_{HA} by the activities of the acid and its negative ion constituent, pK′ may be expressed as a function of the thermodynamic dissociation constant K:

$$pK' = pK + \log \frac{f_A}{f_{HA}} \quad (2)$$

For moderately dilute solutions Hitchcock has proposed an approximation of the limiting law:[7]

$$\log \frac{f_A}{f_{HA}} = B\mu - A\sqrt{\mu} \quad (3)$$

where A is the familiar Debye-Hückel constant and B is a constant independent of the concentration but may vary for different solutes. The preceding expres-

*From the William Hallock Park Laboratory, Bureau of Laboratories, Department of Health, New York.
Received for publication, March 13, 1940.

sion for $\log \frac{f_A}{f_{HA}}$ is substituted into equation (2); pK′ is obtained as a function of the thermodynamic dissociation constant and of an approximate form of the limiting law:

$$pK' = pK - A\sqrt{\mu} + B\mu \quad (4)$$

The following procedure was employed in our work. pK′ values were first computed from the measured pH values of the buffer mixtures by means of equation (1). It was found that by employing the apparent linear relation between the expression $(pK' + A\sqrt{\mu}$ and the ionic strength μ, the constants pK and B in equation (4) could then be evaluated by the Method of Least Squares. pK′ values for the buffer acids computed by means of equation (1) were then compared with pK′ values computed by means of equation (4). The deviations between the two sets of pK′ values for a given buffer acid at a given temperature serve as a measure of the degree of conformity of the pH scale adopted in this work with thermodynamic principles and an approximate form of the Debye-Hückel theory.

The foregoing method was essentially that of extrapolation employed by Bjerrum and Unmack[8] to evaluate the thermodynamic dissociation constant of citric acid and other weak acids at various temperatures.

EXPERIMENTAL

The procedure adopted consisted in the measurement of pH values of buffer solutions set up in a galvanic cell with liquid junction:

Glass, Solution X:KCl (saturated): HgCl, Hg

An Electron Ray Meter was employed in conjunction with a MacInnes-Belcher durable condenser type glass electrode. The latter has been amply described in the literature.[9-11] The Electron Ray Meter is a potentiometer capable of recording either millivolts or pH units. The essential parts of the meter consist of a cathode-ray tube which in conjunction with a triple grid pentode detector and amplifier, operate as null-point indicator. In these determinations the instrument was set to record directly in pH units.

Buffer solutions were prepared with Baker's "analyzed" salts. A 0.05 molar solution of primary potassium phthalate ($KHC_8H_4O_4$) was employed as a primary pH standard. The latter was prepared from the crystalline salt purchased from the National Bureau of Standards. The pH values of this solution have been carefully determined by MacInnes and his associates.[6] Using their work as a basis, the following pH values of 0.05 molar $KHC_8H_4O_4$ were adopted as standards of reference throughout this work:

t°C.	pH
10	4.00
25	4.00
38	4.02

Test solutions were prepared from the buffer mixtures by dilution with appropriate volumes of distilled water or 0.1454 molar sodium chloride solution.

pH measurements were carried out by enclosing the glass electrode filled with the test fluid in an electrically grounded metal case, which in turn was

TABLE I

PHOSPHATE BUFFERS

(1)	(2)	(3)	(4)	(5)				(6)	(7)	(8)	
				pK′						pK″	
% DILUTION	KH_2PO_4	Na_2HPO_4	μ	25° C.		38° C.		NaCl	$\mu_{(1)}$	25° C.	38° C.
				OBSERVED	CALCULATED*	OBSERVED	CALCULATED†				
0	0.02500	0.02500	0.10000	6.86	6.85	6.83	6.83				
10	0.02250	0.02250	0.09000	6.86	6.87	6.83	6.85	0.01454	0.10454	6.84	6.82
25	0.01875	0.01875	0.07500	6.89	6.88	6.86	6.86	0.03635	0.11135	6.82	6.80
50	0.01250	0.01250	0.05000	6.93	6.93	6.90	6.90	0.07271	0.12271	6.78	6.76
75	0.00625	0.00625	0.02500	7.00	6.99	6.97	6.97	0.10906	0.13406	6.75	6.73
90	0.00250	0.00250	0.01000	7.06	7.06	7.03	7.03	0.13087	0.14087	6.72	6.70

*25° C. pK′ = 7.20—1.518 $\sqrt{\mu}$ + 1.34μ.

†38° C. pK′ = 7.18—1.557 $\sqrt{\mu}$ + 1.42μ.

TABLE II

BORAX BUFFERS

(1)	(2)	(3)	(4)						(5)	(6)	(7)	
			pK′								pK″	
% DILUTION	$Na_2B_4O_7$	μ	10° C.		25° C.		38° C.		NaCl	$\mu_{(1)}$	25° C.	38° C.
			OBSERVED	CALCULATED*	OBSERVED	CALCULATED†	OBSERVED	CALCULATED‡				
0	0.05000	0.10000	9.30	9.30	9.18	9.18	9.05	9.05				
10	0.04500	0.09000	9.30	9.30	9.18	9.18	9.05	9.05	0.01454	0.10454	9.15	9.03
25	0.03750	0.07500	9.29	9.29	9.18	9.18	9.05	9.06	0.03635	0.11135	9.14	9.00
50	0.02500	0.05000	9.29	9.30	9.18	9.19	9.05	9.06	0.07271	0.12271	9.11	8.96
75	0.01250	0.02500	9.30	9.30	9.19	9.19	9.08	9.08	0.10906	0.13406	9.08	8.92
90	0.00500	0.01000			9.21	9.21	9.09	9.10	0.13087	0.14087	9.06	8.90

*10° C. pK′ = 9.35—0.493 $\sqrt{\mu}$ + 1.10μ.

†25° C. pK′ = 9.25—0.506 $\sqrt{\mu}$ + 0.93μ.

‡38° C. pK′ = 9.14—0.519 $\sqrt{\mu}$ + 0.74μ.

enclosed in an incubator with thermostatic temperature control. By means of the latter the temperature around the glass electrode assembly (glass and calomel electrodes) was kept constant within ± 0.5° C. during any measurement. When not in use, the glass electrode was filled with distilled water. By this practice the residual potential within the glass membrane (asymmetry potential) is maintained at a low constant level.[10]

EXPERIMENTAL RESULTS

The pH values of the unknown test solutions in the accompanying tables represent the results of at least three concordant measurements at each temperature. For the test solutions between pH 4.0 and 9.0, the MacInnes-Belcher glass electrode in conjunction with the Electron Ray Meter, functioned accurately, well within the limits ± 0.02 pH unit. For test solutions having values ranging from pH 9.0 to 9.3, the accuracy of the instrument was reduced to about ± 0.03 pH unit. Employing the pH values assigned to the standard phthalate buffer, the apparatus yielded pH values for the undiluted buffers which differed from the values obtained by Hitchcock and MacInnes with the hydrogen electrode by ± 0.01 pH unit. However, the pH values of the acetate buffer mixture at 25° C. and that of the borate buffer at 38° C. differed from the values obtained by MacInnes and Hitchcock by ± 0.02 pH unit.[4-6]

The following values of the Debye-Hückel constant A[12] were employed in equation (4):

t°C.	A
10	0.493
25	0.506
38	0.519

The value of A at 10° C. was obtained by graphical interpolation. Between 0° C. and 18° C., A is approximately a linear function of the temperature.

Table I summarizes the results obtained with the phosphate buffer mixtures at 25° and 38° C. Since these solutions consist of 1:1 buffer mixtures, C_A and C_{HA} are equal and the pK′ values computed from equation (1) are numerically equal to the measured pH values of the buffers. Likewise pK″ values are equal to the measured pH values of the saline buffer mixtures. The pH values have, therefore, been omitted from this table.

Column (1) gives the dilution factor or the percentage dilution with distilled water or 0.1454 molar sodium chloride solution. Columns (2) and (3) give the molar concentrations of primary potassium phosphate (KH_2PO_4) and secondary sodium phosphate (Na_2HPO_4), respectively. Column (4) gives the values of the ionic strength of the test solutions. Column (5) gives the observed and calculated pK′ values of the buffer acids by means of equation (1) and equation (4), respectively. Columns (6), (7), and (8) contain data for the buffer solutions diluted with 0.1454 molar sodium chloride solution, which have been included in the table for comparison with the corresponding data in aqueous solution (Table I).

For the phosphate buffer mixtures equation (4) was altered in accordance with the interionic attraction theory by the use of 3A in place of A. The ionic strength of the aqueous buffer solutions was taken as four times the con-

TABLE III

SODIUM ACETATE—ACETIC ACID BUFFERS*

(1)	(2)	(3)	(4)	(5)			(6)			(7)	(8)	(9)		(10)	
% DILUTION	CH_3COOH	CH_3COONa	μ	pH			pK′ (OBSERVED)			NaCl	$\mu_{(1)}$	pH		pK″	
				10° C.	25° C.	38° C.	10° C.	25° C.	38° C.			25° C.	38° C.	25° C.	38° C.
0	0.05230	0.05000	0.05000	4.66	4.68	4.66	4.68	4.70	4.68						
10	0.04707	0.04500	0.04500	4.66	4.68	4.66	4.68	4.70	4.68	0.01454	0.05956	4.68	4.66	4.70	4.68
25	0.03923	0.03750	0.03750	4.67	4.69	4.67	4.69	4.71	4.69	0.03635	0.07387	4.65	4.63	4.67	4.65
50	0.02615	0.02500	0.02500	4.67	4.70	4.68	4.69	4.72	4.70	0.07271	0.09773	4.65	4.63	4.67	4.65
75	0.01308	0.01250	0.01250	4.70	4.72	4.70	4.72	4.74	4.72	0.10906	0.12158	4.64	4.62	4.66	4.63
90	0.00523	0.00500	0.00500		4.76	4.74		4.78	4.76	0.13087	0.13589	4.64	4.62	4.65	4.63

*To compute pK′ from equation (4):

10° C. $pK' = 4.76 - 0.493 \sqrt{\mu} + 0.61\mu$.

25° C. $pK' = 4.78 - 0.506 \sqrt{\mu} + 0.87\mu$.

38° C. $pK' = 4.76 - 0.519 \sqrt{\mu} + 0.52\mu$.

centration of either phosphate salt. The ionic strength of the saline buffer solutions was taken as the molar concentration of sodium chloride and four times the molar concentration of either phosphate salt. The average differences between pK′ values obtained from equation (1) and those computed from equation (4) are ± 0.01 and ± 0.00 at 25° C. and 38° C., respectively.

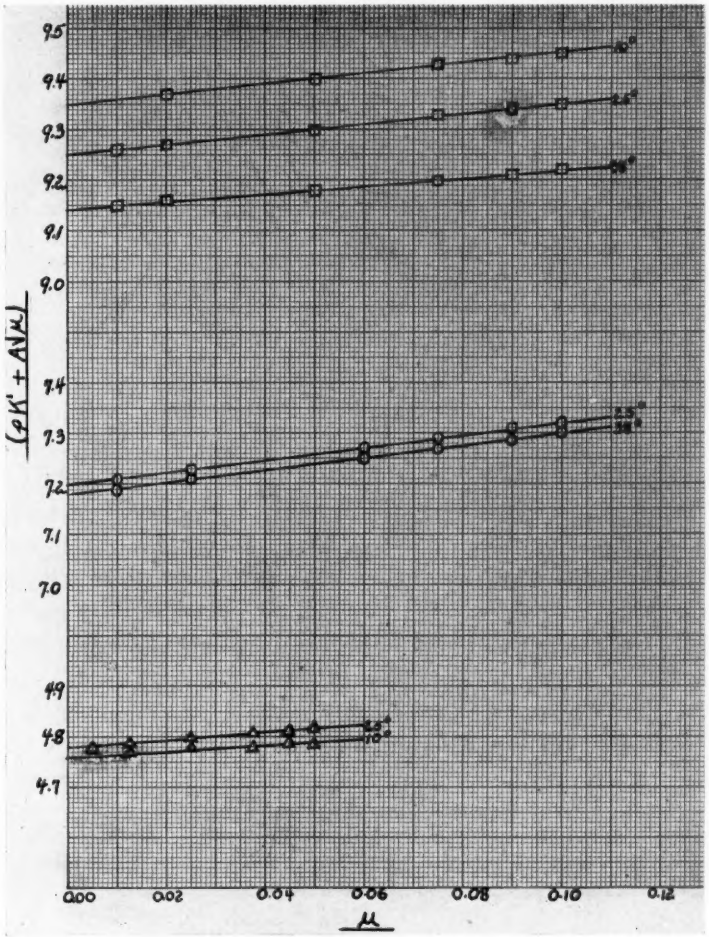


Fig. 1.—Extrapolation of pK,′ data for acetate buffers, triangles; phosphate buffers, circles; and borax buffers, squares.

The data for the borax buffers are summarized in Table II. The following possible paths of the hydrolytic reactions have been proposed:[4]

$$Na_2B_4O_7 + H_2O = 2Na^+ + 2BO^-_2 + 2HBO_2 \text{ (A) or}$$
$$Na_2B_4O_7 + 5H_2O = 2Na^+ + 2H_2BO^-_3 + 2H_3BO_3 \text{ (B)}$$

Hitchcock has shown that neither reaction (A) nor reaction (B) go to completion, as indicated by an actual alkalinity of 2×10^{-5} mols per liter of hydroxyl ions in the borax solutions. He suggests that this may be due to a reaction between the borate ion and water to form undissociated acid. Therefore, C_{HA} was obtained by adding C_{OH} (2×10^{-5} mols per liter) to the molar concentration of sodium borate. Likewise, C_A was taken as equal to the difference between the molar concentration of borax and the hydroxyl-ion concentration.

Since pK′ values computed by means of equation (1) were numerically equal to the observed pH values, the latter have been omitted from the table (Table II).

For the aqueous borax solutions the ionic strength was taken as twice the molar concentration of sodium borate. For the borax buffers diluted with sodium chloride solution, the ionic strength was taken as the sum of the molar concentration of sodium chloride and twice the molar concentration of sodium borate. The average differences between pK′ values obtained from equation (1) and those computed by means of equation (4) are ±0.00, ±0.00, and –0.01 at 10° C., and 25° C., and 38° C., respectively.

Table III summarizes the data for the acetate buffer mixtures. Both the ionic strength of the aqueous buffer mixtures and the concentration of the negative ion constituent, C_A, were taken as equal to the molar concentration of sodium acetate. The concentration of the undissociated acid, C_{HA}, was taken as equal to the molar concentration of acetic acid in the buffer mixtures. The ionic strength of the saline buffers was taken as the sum of the molar concentrations of sodium chloride and sodium acetate.

The average differences between pK′ values obtained from equation (1) and those computed by means of equation (4) were +0.01 at 10° C., 25° C., and 38° C., respectively, for the acetate buffers.

Fig. 1 shows graphically the method of extrapolation employed to evaluate the constants pK and B in equation (4). The points on the lines were obtained by the Method of Least Squares, using the appropriate data in Tables I, II, and III for aqueous solutions of the phosphate, borate, and acetate buffer mixtures. The best straight lines were then drawn through these points (Fig. 1). The intercepts on the $pK' + A\sqrt{\mu}$ axis give the pK values of the buffer acids at infinite dilution. A comparison of limiting pK values obtained in this study with those obtained by others is given in Table IV.

TABLE IV

LIMITING pK VALUES OF SOME WEAK ACIDS AT 10° C., 25° C., AND 38° C.

ACID	t° C.	(6)*	(5)†	(13)	(14)	(15)	(16)
CH_3COOH	10	4.76		4.762			
	25	4.78		4.7572	4.7562		
	38	4.76	4.766				
H_3BO_3	10	9.35				9.389	
	25	9.25				9.237	
	38	9.14	9.143				
H_3PO_4	25	7.20					7.206
(pK_2)	38	7.18	7.19				

*Obtained by the method of least squares from the appropriate data in the present work.

†Interpolation of data of Harned and Ehlers,[13] Owen,[15] and Nims,[16] for 38° C. by Hitchcock and associates.

Table V gives the pH values at various temperatures of standard buffers commonly employed in electrometric pH measurements. These are compared with the pH values obtained by Hitchcock,[4, 5] MacInnes,[6] and their associates at corresponding temperatures, using the hydrogen electrode. In no case do the values obtained in the present work differ from the values obtained by these workers by more than +0.02 pH unit.

TABLE V

COMPARISON OF pH VALUES OF STANDARD BUFFER SOLUTIONS AT VARIOUS TEMPERATURES

BUFFER MIXTURE	t° C.	(G)	(6)	(4) (5)
0.05 M $KHC_8H_4O_4$	10	4.00		
	12		4.000	
	25	4.00	4.000	4.010
	38	4.02	4.015	4.025
0.01 M CH_3COOH + 0.01 M CH_3COONa	10	4.70*		
	12		4.710	
	25	4.72*	4.700	4.714
	38	4.70*	4.710	
0.025 M KH_2PO_4 + 0.025 M Na_2HPO_4	25	6.86		6.855
	38	6.83		6.835
0.05 M $Na_2B_4O_7$	10	9.30		
	25	9.18		9.180
	38	9.05		9.070

(G) Measured pH values obtained in the present work.

*Actual concentrations of sodium acetate and acetic acid used here: 0.01308 M CH_3COOH and 0.01250 M CH_3COONa.

DISCUSSION

Examination of the pH data in the tables shows that the pH values of all the buffer solutions increase with increasing aqueous dilution. While the increases are relatively small for the acetate and borate buffer mixtures, the phosphate buffer suffered a large increase in pH, amounting to 0.2 pH unit at 90 per cent dilution with distilled water. On the other hand, all the buffer mixtures suffered a marked decrease in pH, with increasing dilution with sodium chloride solution. These results are in agreement with those obtained by other workers.[3, 17] Moreover, while increases in temperature affected the undiluted acetate and phosphate buffers least, the borax buffer suffered a decrease of 0.25 pH unit between 10° and 38° C. It has already been pointed out that the pH values of the undiluted buffers showed good agreement with the values obtained by Hitchcock and MacInnes with the hydrogen electrode.

Since it is possible to measure mean ionic activities only, pH as a function of the negative logarithm of the hydrogen-ion activity cannot be placed on a strict theoretical basis. With this fact in mind, any attempt to provide a strict thermodynamic basis for the pH scale is necessarily limited. The efforts of MacInnes and his associates in this direction are noteworthy. Employing extremely dilute buffers, they applied the limiting law to pK data on acetate buffer mixtures at 12° C., 25° C., and 38° C. However, values of the Debye-Hückel constant A were obtained which were greater than those required by theory.[6] They indicate that if pH were a function of mean ionic activities, extrapolation of pK data would yield curves whose limiting tangents would approach the values of A obtained with their data on acetate buffer mixtures.[18, 19]

Since the solutions employed in this work were not sufficiently dilute to warrant the application of the limiting law, an approximation of the Debye-Hückel theory was used. The method of extrapolation applied here gave limiting pK values for the buffer acids which showed fair agreement with the values obtained by other workers, with the exception of boric acid at 10° C. The pK value of boric acid, obtained by the procedure described previously, was about 0.04 unit lower than that obtained by Owen for the same temperature. The values of B in equation (4) varied with the temperature and the type of buffer acid,

SUMMARY

Using the pH values assigned to a standard phthalate buffer as reference, the pH values of acetate, phosphate, and borate buffers diluted with water and with sodium chloride solution have been determined at 10° C., 25° C., and 38° C. with a glass electrode in a cell with liquid junction. pK′ and pK″ values have been computed from the appropriate pH data for the aqueous and saline buffer solutions, respectively. Employing the apparent linear relation between $(pK' + A\sqrt{\mu})$ and μ, limiting pK values of the buffer acids have been computed. Tests have been made of the degree of conformity of the pH scale adopted in this work with the limiting pK values and an approximation of the Debye-Hückel limiting law. Within the limits of experimental error the MacInnes-Belcher glass electrode, in conjunction with the Electron Ray Meter, gave accurate and reproducible pH values which were in agreement with the values obtained by others with the hydrogen electrode.

REFERENCES

1. Glaubiger, A., and Falk, K. G.: The Determination of pH Values of Biologic Fluids. I, J. Lab. & Clin. Med. **25**: 369, 1940.
2. Glaubiger, A.: The Determination of pH Values of Biological Fluids. II. The Influence of Temperature, Dilution, and the Type of Diluent Upon the pH Values of Bacteriologic Media, J. Lab. & Clin. Med. **25**: 1311, 1940.
3. Clark, W. M.: The Determination of Hydrogen Ions, ed. 3, Baltimore, 1928, Williams & Wilkins Co.
4. Hitchcock, D. I., and Taylor, A. C.: The Standardization of Hydrogen Ion Determinations. I. Hydrogen Electrode Measurements With Liquid Junction, J. Am. Chem. Soc. **60**: 1812, 1937.
5. Hitchcock, D. I., and Taylor, A. C.: The Standardization of Hydrogen Ion Determinations. II. A Standardization of the pH Scale at 38°, J. Am. Chem. Soc. **60**: 2710, 1938.
6. MacInnes, D. A., Belcher, D., and Shedlovsky, T.: The Meaning and Standardization of the pH Scale, J. Am. Chem. Soc. **60**: 1094, 1938.
7. Hitchcock, D. I.: The Extrapolation of Electromotive Force Measurements to Unit Ionic Activity, J. Am. Chem. Soc. **50**: 2076, 1928.
8. Bjerrum, N., and Unmack, A.: Elektrometrische Messungen mit Wasserstoffelektroden in Mischungen von Säuren und Basen mit Salzen, Kgl. Danske Videnskab. Selskab. Math.-fys. Medd. **9**: 1, 1929.
9. MacInnes, D. A., and Belcher, D.: Further Studies on the Glass Electrode, J. Am. Chem. Soc. **53**: 3315, 1931.
10. MacInnes, D. A., and Belcher, D.: A Durable Glass Electrode, Indust. & Engin. Chem. Anal. Ed. **5**: 9, 1933.
11. MacInnes, D. A., and Longsworth, L. G.: The Measurement and Regulation of pH With the Glass Electrode, Tr. Electrochem. Soc. **71**: 73, 1937.
12. MacInnes, D. A.: Principles of Electrochemistry, New York, 1939, Reinhold Publishing Co.
13. Harned, H. S., and Ehlers, J. W.: The Dissociation Constant of Acetic Acid From 0° to 35°, J. Am. Chem. Soc. **54**: 1350, 1932.
14. MacInnes, D. A., and Shedlovsky, T.: The Determination of the Ionization Constant of Acetic Acid at 25° From Conductance Measurements, J. Am. Chem. Soc. **54**: 1429, 1932.
15. Owen, B. B.: The Dissociation Constant of Boric Acid From 10° to 50°, J. Am. Chem. Soc. **56**: 1695, 1934.
16. Nims, L. F.: The Second Dissociation Constant of Phosphoric Acid From 20° to 50°, J. Am. Chem. Soc. **55**: 1946, 1933.
17. Kolthoff, I. M., and Bosch, W.: The Influence of Neutral Salts on Acid-Salt Equilibria. I. A Contribution to the Knowledge of the Standard Value Used for Calculating the Activity Exponent pH of the Hydrogen Ion Concentration, Rec. Trav. Chem. **46**: 430, 1927.
18. Brown, A. S., and MacInnes, D.: The Determination of Activity Coefficients From the Potentials of Concentration Cells With Transference, J. Am. Chem. Soc. **57**: 1356, 1935.
19. Shedlovsky, T., and MacInnes, D. A.: The Determination of Activity Coefficients From the Potentials of Concentration Cells With Transference, J. Am. Chem. Soc. **59**: 503, 1937.

MEDICAL ILLUSTRATION

MOULAGE PROSTHESIS

CARL DAME CLARKE, PH.D., BALTIMORE, MD.

WITH ILLUSTRATIONS BY JOHN T. STRINGER, JR., AND CARL DAME CLARKE

THE art of face and body restoration is not new. In fact, the Hindus developed methods for the repair of nasal defects which today are considered good operative procedure in rhinoplasty. This was only one of their difficult plastic feats. According to Kazanjian, Rowe, and Young,[1] the literature of plastic surgery dates back as far as Celsus (25 B.C.) and Galen (A.D. 131-201). They call attention to Paul of Aegina (A.D. 630) and Branca (1442). They also mention Ambroise Paré (1541), Tagliacozzi (1546), Pierre Fauchard (1728), Bourdet Suerson and Delabarre (1820), Hullihan, Kingsley, Claude Martin, Nelaton, and Zeis (1900), and others. In 1875 Martin was the first to conceive of immediate prosthesis.

From the prewar plastic surgeons, Morestin, Lexer, and others, it will be noted that the medical and dental literature contains many articles, methods, and case reports concerning surgical and mechanical means of correcting pathologic deformities of the face. The horrible maiming effects of the first World War produced innumerable patients for surgeons, dentists, and moulage workers to treat. It was during war times that the art and science of prosthesis took gigantic strides forward.

In consideration of the fact that another World War is now in progress, it is obvious that there will be hundreds, if not thousands, of cases demanding prosthetic appliances.

In 1938 I published a book on the subject of *Molding and Casting*[2] in which I discussed previous work in moulage prosthesis in detail, as well as methods in present-day use for producing these prostheses. Since that time there have appeared on the market many synthetic plastics which lend themselves readily to the making of prostheses. It is my desire to describe herein the methods for making both a moulage prosthesis on what would ordinarily be a very simple case and a prosthesis for a more advanced case. Such work definitely falls on the scientific illustrator as well as the physician. The formulas for prosthetic work may be compounded by the worker, or the compositions may be purchased already prepared.

The physician can make a single mold and cast of the part to be reconstructed if the case is a simple one. From this mold he can make a reconstruction in wax of the missing part. Practically any dental laboratory can repro-

duce a synthetic plastic duplicate from this wax original. False teeth are made in the same manner by the dental laboratory from a mold supplied by the dentist.

Above all else the case in mind for reconstructive procedure should be considered carefully before any work is begun. The worker should determine whether it is more suitable for treatment by plastic surgery or by reconstructive prosthesis. It should be clearly understood that while the result of surgery may be more satisfactory, its application may involve danger and pain that is distributed over a period of weeks, with uncertain and often mortifying results. The prosthesis, while not a permanent cure, is without danger and involves no pain to the patient either in the making or in the wearing. For patients unable to withstand the debilitating effects of operative procedure, a prosthetic appliance is strongly recommended. I do not advocate the use of a prosthesis in preference to plastic surgery for the correction of facial defects. The case in question must be the deciding factor. However, it cannot be stressed too forcefully that plastic surgery alone should not be considered in every case.

Lederer[3] writes that skin cannot be molded in the manner of clay; hence it is very difficult to produce a lifelike nose or ear by plastic surgery. The nose is constantly under the scrutiny of others, and it stands to reason that the patient would be no worse off without his nose or perhaps with a small adhesive dressing than he would with a few clumps of grafted skin, which at best would still be extremely conspicuous. Lederer maintains further that he, as well as other plastic surgeons, has been disappointed in the net results of his painstaking labors and, in a measure, feels guilty for having subjected his patients to operations which not only brought them great economic loss and suffering, but in many instances jeopardized their health and lives. They had scars remaining where tissue was grafted, spent many uncomfortable weeks in a cast (when Italian grafts were utilized), lost time from their work, and in some instances had to accept primary failure owing to poor healing.

Van Dijk[4] found that the ears he formed by plastic surgery were too bulky and not sufficiently erected, and that the fossa scaphoidea and the anthelix were not built up. He planned in his next patient to get the ear more erect through implants of cartilage, thus giving it a more shell-like appearance.

More and more the surgeon is beginning to recognize a substitute in the form of a prosthesis for certain plastic procedures. These two phases of the healing art are inseparably related. The future of restorative work, just as in the field of prevention, lies in the closest cooperation between physician or surgeon and the prosthetist.

Furthermore, if the surgeon is working with a prosthetist, the necessity of adequate surgical preparation for the application of an artificial restoration cannot be too strongly stressed. The failure of many prosthetic attempts is often caused by the unfortunate necessity of adapting restorations to areas of soft tissue which have been distorted and contracted by unessential

scar tissue and improper postoperative procedure. The success of a prosthetic restoration is in a great measure increased as the degree of mutilation is lessened.

The ability of the surgeon and of the moulage worker has much to do with the final result in either case. I am unable to criticize surgical technique but I am more familiar with the procedure of moulage making and the final prosthetic appliance. In my opinion these prostheses suffer from the same defects that are prevalent in most medical art work. In short, they lack artistic skill in execution and naturalness in its simplest form. The artificial appliance to the human face should, above all else, look realistically natural and individually suitable. The conspicuous errors of most poor prostheses are generally the result of a lack of artistic ability and appreciation on the part of the surgeon or prosthetist. These difficulties may be overcome by having the prosthetic appliance made by a skilled prosthetist in close cooperation with the plastic surgeon. Because the surgeon or dentist has considerable ability in surgery or moulage making does not necessarily mean that he is an artist. His ability or judgment to choose the correct feature in size and relative proportions to be fitted next to other adjoining features of flesh and blood may be lacking.

Another eventual stumbling block to the surgeon and dentist is the final coloring of the artificial part. It takes considerable experience in handling pigments and a natural color sense to blend perfectly the many different hues into the prosthesis to match the surrounding tissue. Hair, bristle brushes and airbrushes, pigments and pastes are often used for this purpose. The artist possessing this color sense yearns to "touch up" the average monochromed prosthesis which in its single hue is not natural. He desires further to eliminate the many false tones and shades of a modernistic pictorial nightmare. For the making of the best facial prosthesis close cooperation between the physician, dentist, and prosthetist is essential. Even the physician having little or no artistic ability can make acceptable prostheses of simple cases, provided he works in close cooperation with a dental laboratory.

In a simple case of prosthesis the first step is to obtain a mold or impression of the defective part. An agar composition is generally used for such work. This molding material may be purchased under the trade name of "Hydrocolloid" (Detroit Dental Mfg. Co.), "Dentocoll" (L. D. Caulk Co.), and others. A serviceable composition may be prepared from one of the formulas published in the articles listed in the references. If the worker is interested only in the results he obtains rather than in the materials he uses, he will find it more desirable to purchase the composition already prepared. The commercial preparation comes in a handy collapsible tube which is placed in boiling water for ten minutes. It is then transferred to lukewarm water and allowed to cool to a point where the tube does not feel uncomfortable when placed on the bare wrist. An agar composition should never be applied when it is so warm that it would be uncomfortable to the patient. This material does not set until it reaches a point (42° C.) slightly above body temperature (37.8° C.). Even though the patient can withstand the heat, the material must

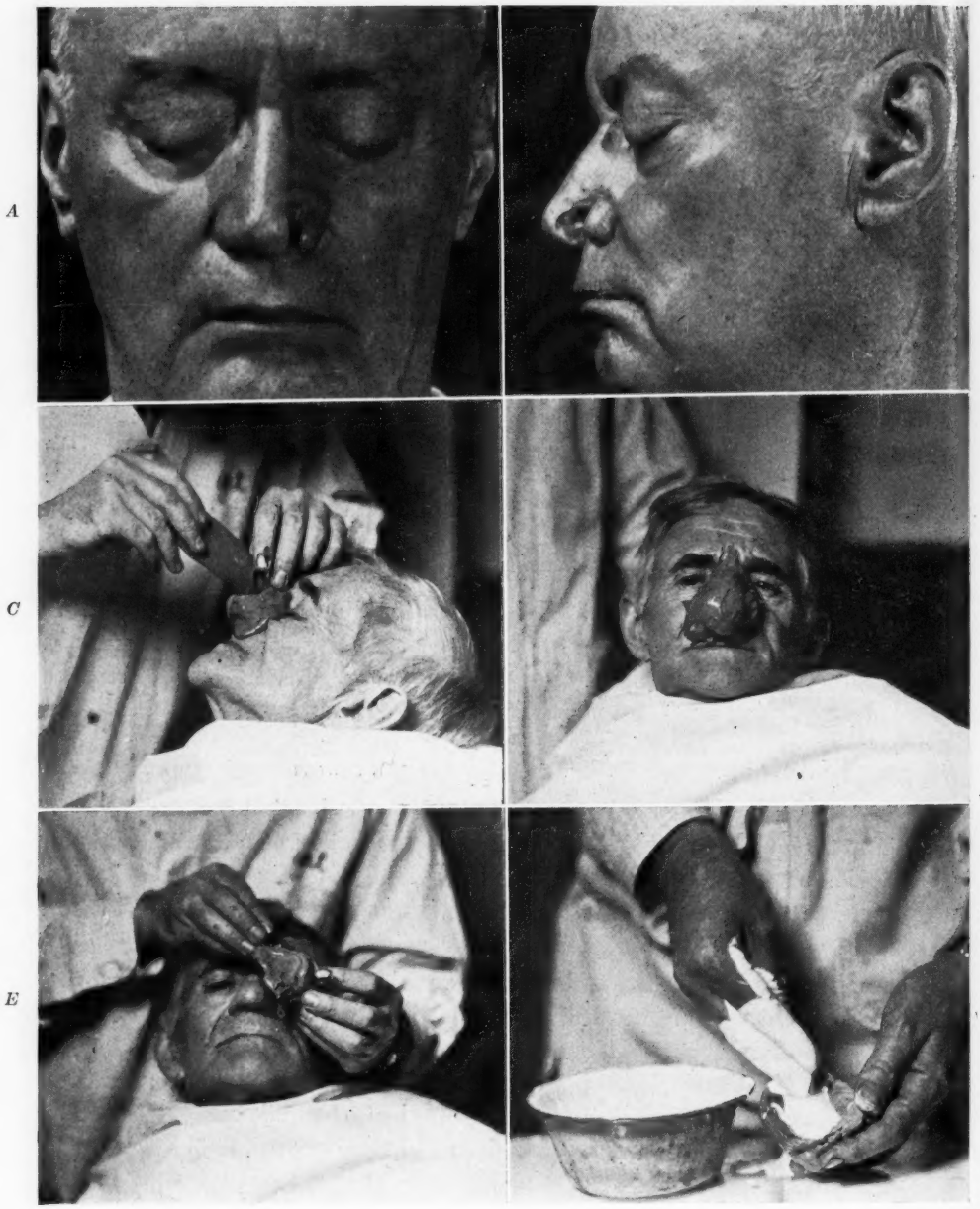


Fig. 1.—*A*. Front view of a defective area on the nose.
B. A side view of the same area.
C. The warm agar composition being applied to the nose directly from the tube.
D. A front view of the agar mold in place just after setting.
E. The agar mold being removed.
F. The agar mold being filled with plaster of Paris, which sets to form the positive.

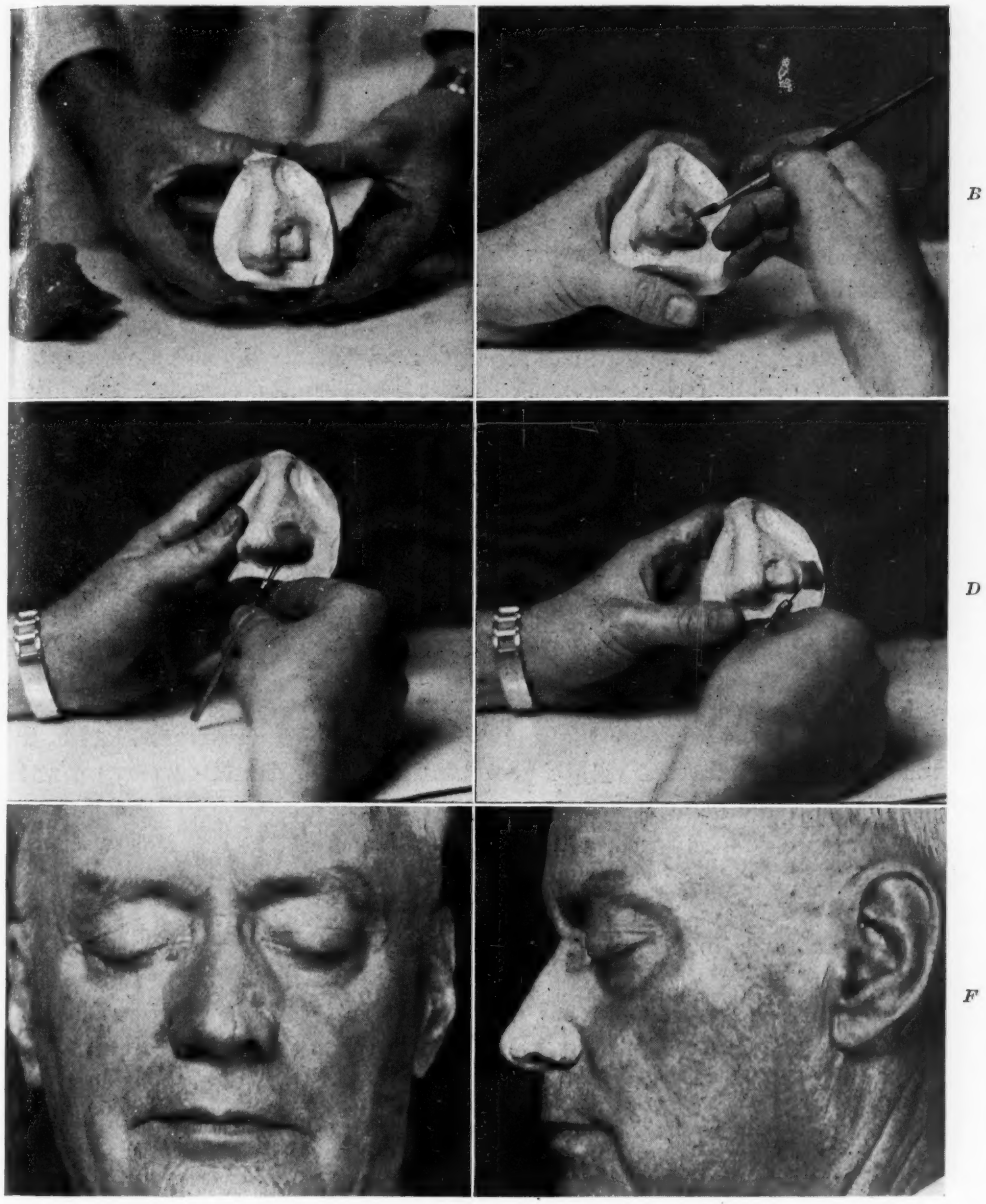


Fig. 2.—*A*. The plaster impression after removal from the agar mold. The agar mold can be seen to the left. Part of the defect has been filled with additional plaster to allow a space for breathing. This is done with a soft brush and a plaster-water mixture.

B. The remainder of the defective area is filled with a wax composition (the formulas for which are in the text). A brush is used for applying the hot wax mixture. This is continued until the area is filled with the wax.

C. The wax is then carved down with an ordinary scalpel and shaped to follow the natural contours of the ala of the nose. A sharp, pointed probe is inserted into the wax from the inner side and the wax is removed. This wax will not adhere strongly to the wet plaster.

D. Showing the wax prosthesis being removed from the plaster nose.

E. The wax is then fitted onto the patient's nose. If it fits perfectly it is turned over to a dental laboratory for reproducing into one of the modern synthetic plastics. A plastic is chosen to match the skin of the patient. This completed prosthesis is held in place by a solution of gum mastic dissolved in equal parts of alcohol and ether.

F. A side view of the patient with the prosthesis in place. *E* and *F* show the patient without any cosmetics over the prosthesis. The false area may be made to blend more perfectly with the skin by the use of "Covermark" or a similar cosmetic.

remain in place longer for sufficient cooling or setting time. When the tube has sufficiently cooled, its end is cut with a scissors and the material is squeezed over the afflicted part (Fig. 1*c*). In ten minutes the agar composition sets, after which it is removed (Fig. 1*e*). This mold should not be removed until it definitely feels firm. An entire ear can be cast in the same manner in a one-piece mold. This mold can then be filled with either hot wax or plaster of Paris. Wax should be used in making an ear as an intermediate step for a final rubber or synthetic plastic prosthesis. However, in considering the patient in the illustration (Fig. 1), it is best that the nose in this case be made in plaster of Paris, for the defective part must be modeled in wax on the plaster. This is done because the wax will separate readily from the wet plaster.

After the plaster nose has set, it is removed from the agar mold (Fig. 2*a*). Additional plaster is applied with a soft brush to the area represented by the opening in a normal nose for breathing. This will create the opening for breathing in the prosthesis. A brush is then dipped in a hot wax composition and applied directly over the defective area (Fig. 2*b*).

A wax composition suitable for this purpose is as follows:

	Parts by weight
Paraffin (62° C.)	10
Rosin	8
Carnauba wax	1

A ready-made wax may be purchased from dental supply stores. After this wax has set, it is carved with a pocketknife, scalpel, dental or sculptor's tool until it takes the shape of a normal nose (Fig. 2*c*).

A sharp, pointed probe is then inserted from the inner surface of the wax ala and with a slight pressure it should separate from the wet plaster (Fig. 2*d*). This wax piece can be tested on the patient (Figs. 2*e* and *f*). If it fits perfectly it is turned over to a dental laboratory to be made into one of the synthetic plastics, such as "Vernonite," "Crystalex," and "Lucetone." Of course, a shade should be chosen which matches the patient's skin as nearly as possible. If in the wax stage the prosthesis does not fit, it can be adjusted or changed so that it will fit the patient.

It is not advisable for the average physician or surgeon to attempt to do the final stage in the making of a prosthesis, since the dental laboratory is more fully equipped for such work and its charges are reasonable. After the synthetic plastic part is finished, the edge that comes in contact with the skin is painted with a solution of gum mastic dissolved in equal parts of alcohol and ether. The prosthesis is then put in place and held there with a finger until the solvent has evaporated and caused the gum mastic to hold it in place. This takes less than a minute in most instances.

A more complicated case (Figs. 3, 4, and 5) requires a greater knowledge of making prosthetic appliances. This case was a patient of the late Dr. A. Y. Russell, whom I assisted in developing the prosthesis.

The first procedure was to obtain a plaster cast of the face in its original condition. Tubes were inserted in the nasal cavity and gauze was placed around

the tubes. The mold was then made of agar and reinforced with a plaster shell or mother mold (Fig. 3*c-f*). From this mold the plaster positive or cast which showed the complete facial defect was made. In this case ordinary gray plasteline was used to model a nose on the plaster case (Fig. 4*b*). This, of course, requires artistic ability or training. However, the modeling of a nose is not essential, for a mold may be made of a living person who has a well-formed, if not an idealistic, nose. This person must be of the same type and general proportions as the patient. The cast from this second mold may be trimmed to fit into the open space on the plaster mask of the patient. The edges between the two casts may then be smoothed out by the use of plasteline.

From the modeled nose a mold was made of the reconstruction only (Fig. 4*c*) and a red wax impression was made from the mold (Fig. 4 *c-g*). The red wax cast is later removed from a piece mold; it sometimes requires boiling water to melt it. The subject of agar and wax compositions has been discussed.

Upon its removal from the mold (Fig. 4*c*), the back of the cast was modeled or carved into the shape (Fig. 4*d*). An extra knob was added to the wax impression as a support for attachment to the artificial palate which had been made previously.

After the wax impression was modeled or carved to the desired shape to suit the mechanical necessities of the cast, a piece mold of the entire cast, including front and back, was made (Fig. 4*c*). From this mold other red wax casts seen in *d* could be made. This is the same piece mold that is to be used to cast the final impression, provided the red wax cast fits the patient. Tests to determine the snugness of the fit were made on the original plaster cast of the face after the plasteline nose had been removed. Such tests were also made by putting the wax impression on the patient (Fig. 4*f* and *g*). The final impression was then cast.

The material used for this final cast should be considered seriously. Numerous substances such as gelatin (Henning's method, described by Salamon; also elaborated on by Zinsser, Pont, Bercowitsch;[5] Kazanjian, Rowe, and Young[1]) and light metals, such as aluminum, have been used. Plastics, such as vulcanite or vulcanized rubber (not vulcanized latex) and celluloid compositions have been employed as positive casting materials.

The materials used in the past have been discussed fully in the articles listed as references. In my opinion the best materials for making a prosthesis to be worn on the face are the synthetic plastics previously discussed, and vulcanized latex. In comparison with rubber the synthetic plastics have one serious drawback when they are considered for large prostheses as, for example, an entire ear; they are hard and rigid, and somewhat heavier than rubber.

In making a moulage prosthesis of rubber, vulcanized latex, powdered fillers, and coloring agents are used. First, the powder is added to the flesh-colored latex until the desired consistency is obtained. Permanent water-soluble aniline dyes having a neutral or alkaline reaction are used for coloring the vulcanized latex to a flesh tint. The latex is then poured into a plaster mold and allowed to remain until the plaster absorbs enough water from the vulcanized latex to

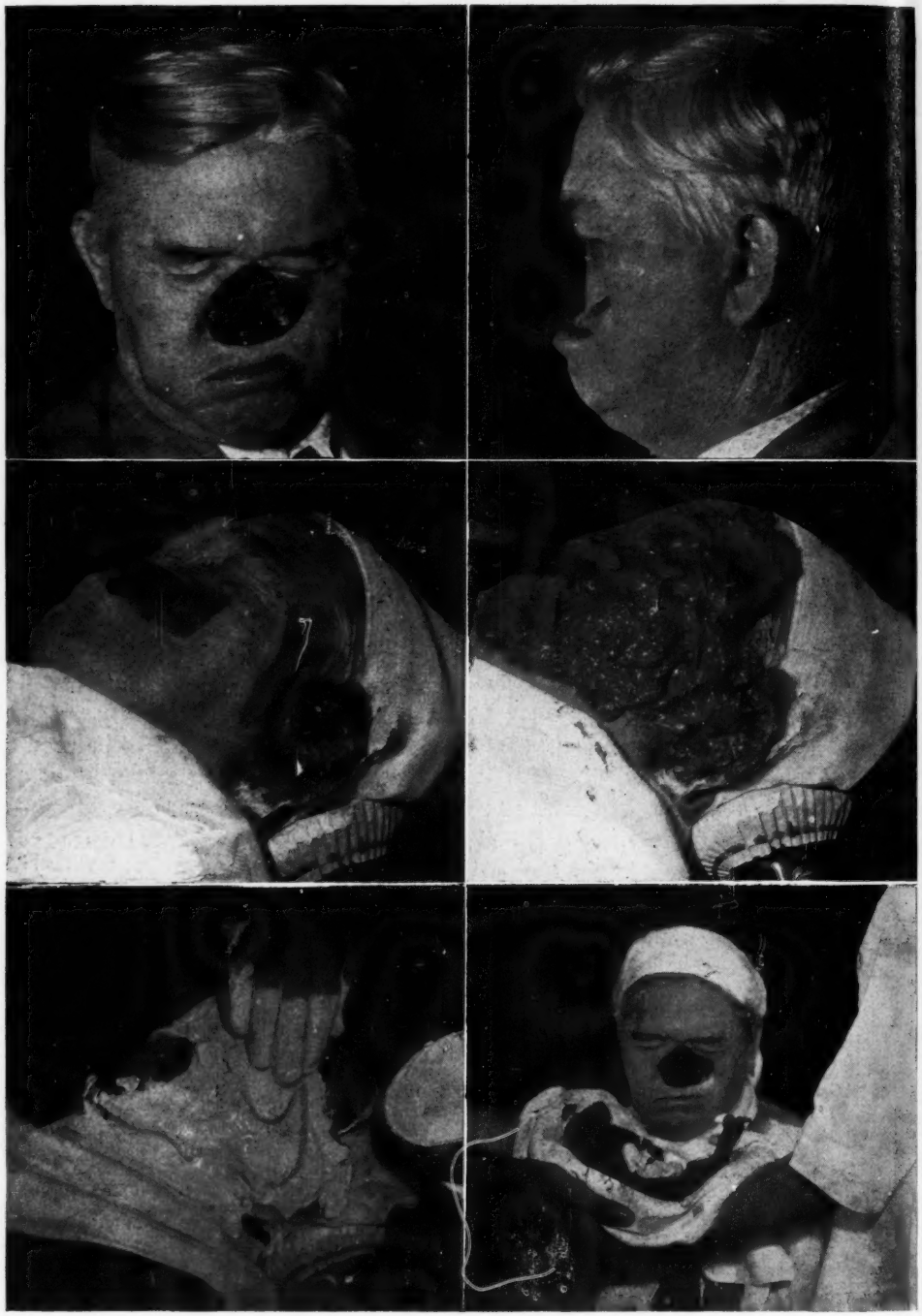


Fig. 3.—*A*. Front view of a patient showing the healed lesion of a carcinoma. An artificial hard palate has been made and put in place.

B. Side view of the same patient.

C. Rubber tubes are inserted into the opening to facilitate breathing. Sterile gauze is packed around these tubes. Agar is used to make the mold. Some of this material was used in the illustration to cover the gauze.

D. The agar mold near completion.

E. The agar mold is reinforced with plaster.

F. The removal of the two molds by gravity. The gauze that was used to fill the cavity in the face can be seen.

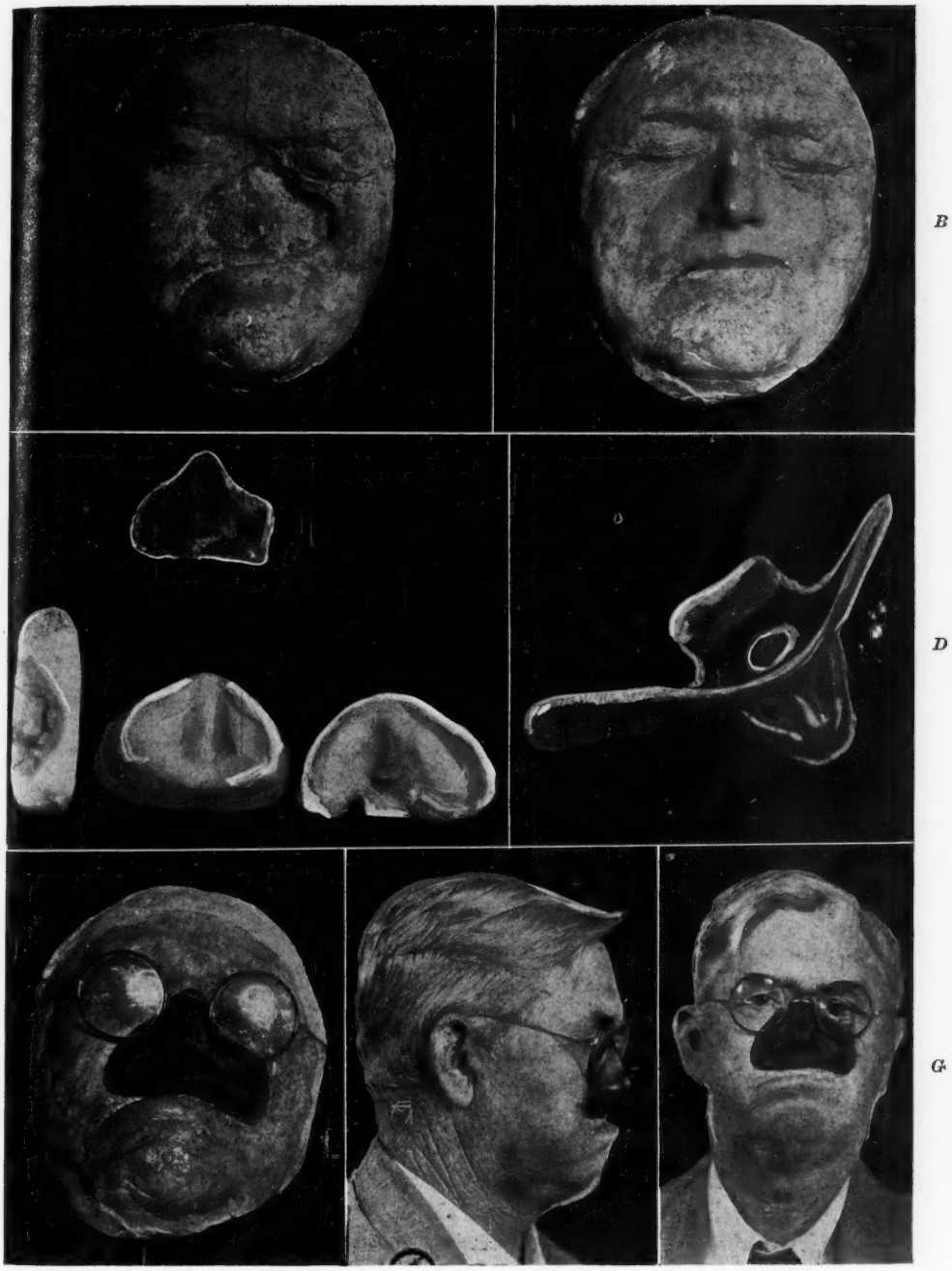


Fig. 4.—*A*. The plaster impression of the face from the agar mold.

B. A nose is modeled in plasteline on the plaster cast. A plaster impression is made of the plasteline model, from which a red wax nose is cast.

C. While the red wax is still in place the back of the red wax is molded to form a squeeze piece mold.

D. The underside of the red wax nose.

E. The wax nose is tested with the glasses on the plaster cast.

F. It is then fitted to the patient.

G. Front view of the patient with the red wax nose in place.

cause a deposit of sufficient thickness. The surplus is then poured out. The latex can also be painted, layer by layer, into an agar, wax, or metal impression of a duplicate of the part to be replaced. Each layer of the rubber composition sets within a few minutes, after which another layer is applied. A celluloid solution may be poured or painted over this rubber to give it additional support and serve in a manner similar to the cartilage in the ear. The liquid celluloid may be made by dissolving scrap celluloid in butyl acetate. After the celluloid has set, more rubber may be painted over it, thus incasing this inflammable material in rubber. In other words, the rubber takes the place of flesh and the celluloid takes the place of cartilage. After drying, the resulting rubber cast is removed from the mold. Gentle heat speeds the setting time of such rubber casts.

It is far more difficult to trim and patch rubber impressions than to do the same work on wax or plaster impressions. Such trimming as is necessary must be done with very sharp wet knives. A wet blade cuts rubber more easily than a dry blade. Because of the difficulty of patching rubber positives, as many corrections as possible should be made on the mold. Another method is to obtain a thoroughly trimmed and patched wax cast of the individual, and make a second mold over this impression in any metal, except copper or zinc, by electrodeposition of the metal. Nickel may be electroplated on the wax, then copper, and then nickel again, thus incasing the copper between two layers of nickel. The metal mold is then used for casting rubber impressions. In most cases an unlimited number of positives may be obtained from a single mold.

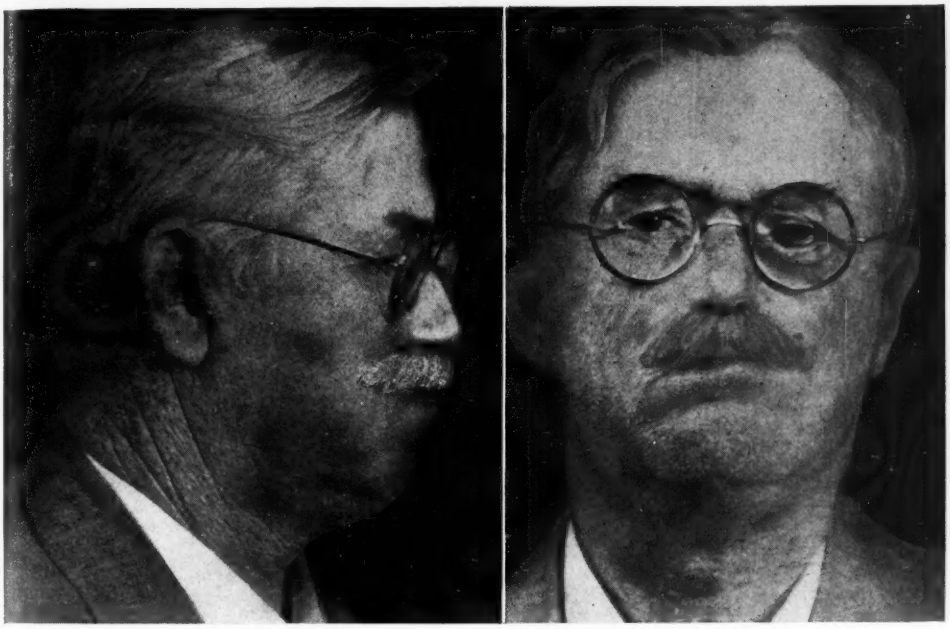
In making a prosthesis of a hand, agar composition can be used to secure a one-piece mold. Wax is poured into the mold to obtain a wax positive. A one-piece plaster mold is made over the wax, and the wax is removed from the plaster with boiling water. Vulcanized latex is poured into the one-piece dry plaster mold to obtain a rubber hand. By this method even the pores of the skin are duplicated.

Vulcanized latex, which is sold under the trade name of "Vultex,"* is undoubtedly one of the best materials to use for making large artificial parts to be worn on the human body. It has many advantages: (1) Vulcanizing agents are unnecessary. (2) It sets in a closed plaster mold into which it may be poured through a funnel-like opening. (3) A separating medium is not necessary, as vulcanized latex does not stick to plaster of Paris. In fact, the plaster absorbs the water from the mixture and causes it to set. (4) Colors, such as carmine (acid-free red water color in tubes), may be incorporated in the vulcanized latex to give it a flesh tone before it is poured. There is enough yellow in the set Vultex to give it a fleshlike tone when the carmine is added. Zinc oxide may be added to the liquid vulcanized latex to prevent the resulting prosthesis from becoming a too dark yellow. (5) Shrinkage does not occur as much in highly concentrated vulcanized latex mixtures as in thin mixtures. Unless the mold is tamped or jarred during the pouring, the resulting prosthesis may be partially hollow. Fillers can be added to make the mixture thicker.

*This material may be obtained from R. W. Edmonds, Manufacturers' Agent, 401 Water Street, Baltimore, Md.

These increase the tendency toward a hollow casting. Of course, large prostheses, such as hands, should be hollow.

Should light metals, such as aluminum and silver or their alloys, be chosen for the prosthesis, the metal may be shaped to the correct form in a swedge mold or die and counter die. These may be of plaster. The formation of the metal must be taken in easy stages, with numerous counterdies of different levels to prevent tearing of the metal. The metal is then annealed between each stage.



A *B*

Fig. 5.—*A*. If the red wax nose fits perfectly the same mold used for casting the red wax may be used for casting the final positive material. In this illustration the final cast has been attached to the spectacles. An artificial mustache has been attached to the final cast.

B. A front view of the same patient.

Metal prosthesis is considered inferior to other substances for attachment to the human body. However, should the worker care to pursue this subject he may find valuable information in the dental literature.

I have discussed moulage prosthesis more fully in the book, *Molding and Casting*. However, since the publication of this book the Mayo Clinic has published two articles on the subject[6, 7] which give specific formulas for compounding rubber prostheses from vulcanized latex. I find that a single formula does not fit every case. If the worker understands his materials, he is likely to vary them to fit individual cases.

The final prosthesis is fixed to the patient with the gum mastic solution previously mentioned or with some mechanical device as eyeglasses or eyeglass frames. The prosthesis in Fig. 5 was riveted to the nosepiece of the eyeglass frame.

Sometimes it is desirable to touch up a prosthetic appliance with additional color because its monochrome effect may not be entirely realistic. If the original

is in celluloid, a small amount of transparent oil color (phototinting color) may be mixed with acetone to be used in the coloring. This acetone softens the celluloid sufficiently to allow some of the dye to penetrate the surface without destroying the skin effect. If a synthetic plastic is used, its solvent can be incorporated with a transparent oil color in the same manner to give additional tints. If the prosthesis is in rubber, benzene or carbon tetrachloride may be incorporated with the transparent oil color. These solvents cause the prosthesis to be slightly sticky immediately after they are used, but they dry in a short while. The worker should realize that to do this work successfully a certain amount of practice and skill is necessary. He should test his tinting ability on objects that have no value. It is possible that his first attempts at prosthetic work will be failures. The objects should be saved to be used in testing other processes in the work, such as tinting.

After the prosthesis is put in place on the patient, cosmetics can be used successfully in making the prosthesis blend with the surrounding tissue. The preparation called "Covermark"* is excellent for this purpose. Any small cracks between the prosthesis and the surrounding tissue may be filled with a putty made by mixing vanishing cream and talcum powder. However, if such cracks are quite obvious it is logical that the prosthesis should be made over. A well-made prosthesis should not be detected at a distance of three feet under ordinary lighting conditions, such as daylight in the average room or artificial light at night.

REFERENCES

1. Kazanjian, V. H., Rowe, A. T., and Young, H. A.: Prosthesis of the Mouth and Face, J. Dent. Research **12**: 651, 1932.
2. Clarke, C. D.: Molding and Casting, Its Technique and Application, Baltimore, 1938, John D. Lucas Co.
3. Lederer, F. L.: Prosthetic Aids in Reconstructive Surgery About the Head, Arch. Otolaryng. **8**: 531, 1928.
4. Van Dijk, J. A.: Acta oto-laryng., Part 1.
5. Bercowitsch, G. G.: Facial Restoration, Dental Cosmos **70**: 167, 1928.
6. Bulbulian, A. H.: An Improved Technic for Prosthetic Restoration of Facial Defects by Use of a Latex Compound, Proc. Staff Meet., Mayo Clin. **14**: 433, 1939.
7. Bulbulian, A. H.: Prosthetic Restorations of Facial Defects by Use of Latex Compound: A Further Detailed Description of the Technic Used, Proc. Staff Meet., Mayo Clin. **14**: 721, 1939.

*Lydia O'Leary, 551 Fifth Avenue, New York City.

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

PURPURA, The Present Status of Thrombocytopenic, Wiseman, B. K., Doan, C. A., and Wilson, S. J. J. A. M. A. 115: 8, 1940.

No single theory for the production of essential thrombocytopenic purpura adequately accounts for all the known facts relating to this disease; however, that of splenic thrombocytolysis appears to violate the fewest.

In differential diagnosis aspiration of bone marrow to rule out leucopenic leucemia, aplastic anemia, pernicious anemia, and neoplasia is at times essential. Historical facts relating to drugs and present or recent contact with certain infectious diseases are most important.

In essential thrombocytopenia the only measure known that will restore the platelet level is splenectomy. The more pronounced the bleeding tendency the more urgent the indication for surgery.

No case of symptomatic thrombocytopenia, regardless of the degree of bleeding, should be submitted to surgery.

LEAD, Absorption and Intoxication in Man Unassociated With Occupational or Industrial Hazards, Hansmann, G. H., and Perry, M. C. Arch. Path. 30: 226, 1940.

The analysis of the data obtained may be divided into facts derived from the data and suggestions for consideration contained in the data.

A. Facts derived from the data:

Examinations of tissues for lead absorption were made on 48 bodies, the ages of which ranged from 11 weeks' gestation to 93 years of age.

The amount of lead in the ribs varied from 23.058 mg. to 0.00 mg. per hundred grams.

The amount of lead in the liver varied from 21.033 mg. to 0.00 mg. per hundred grams.

The lead content is expressed in milligrams per hundred grams of dried tissue.

There appeared to be no relationship between the amount of lead absorbed and the age, except that those subjects containing no lead were either fetuses or children under 12 years of age.

The amount of lead in the liver may exceed that in the rib during prolonged metabolic disturbances, severe infections, and less acute progressive fatal illness.

A mother who has absorbed lead will excrete increasing amounts of lead during gestation, the excretion of which parallels the skeletal growth of the fetus.

The analysis of entire fetuses, the ages ranging from 11 to 24 weeks' gestation, revealed lead in 62.5 per cent of them. The amount in 25 per cent of these bodies may be considered hazardous.

Eighty per cent of fetuses from 4½ months to term had lead in the rib or the liver or in both organs.

All subjects over 12 years of age or 90 per cent of those between birth and 93 years of age revealed evidence of lead absorption.

A review of the results recorded in the literature with which our results were integrated, has established by spectrographic, colorimetric, and titrametric methods that lead absorption is a normal consideration.

The cases were all drawn from Milwaukee and its vicinity.

B. Suggestions for consideration contained in the data:

Lead absorption may act as an unrecognized factor in various diseases in persons who cannot trace their exposure to lead.

Aged persons, who frequently lose, often quite rapidly, upward of 50 per cent of their skeletal calcium, may suffer symptoms of intoxication from the lead which is concurrently released, provided the skeleton is heavily leaded.

Women may suffer from lead intoxication during gestation, owing to the fact that much lead is released from a heavily leaded skeleton during gestation. This may be responsible for some of the anemias closely resembling pernicious anemia which occur during and shortly after pregnancy.

The fetus is likewise exposed to lead hazards, which may result in intoxication of the fetus or expulsion of the fetus as a result of the action of lead on the uterus.

The patient with a heavily leaded skeleton may suffer from symptoms of lead intoxication during uncontrolled metabolic diseases, severe infections, or prolonged, progressive illness, owing to the fact that lead is mobilized from the skeleton and fixed by the organs.

Lead may be an important cause of abortion during the first three months of gestation.

The development of the fetal skeleton may protect the fetus by withdrawal of lead from the circulation.

Lead absorption may become an individual problem and the concern of every physician.

JAUNDICE, Renal Lesions Associated With Deep, Ayer, D. Arch. Path. **30**: 26, 1940.

In infants with long-sustained uncomplicated jaundice, due to congenital atresia of the bile ducts, renal lesions occur without producing oliguria. These lesions consist of focal exudative changes, obstruction of tubules by casts, and phagocytosis of cast material by epithelial cells. These lesions are duplicated in the kidneys from patients with transfusion reaction and the hepatorenal syndrome.

SULFANILAMIDE, A Micro Bedside Test for Determination of, in Body Fluids, Schoeffel, E. W. J. A. M. A. **115**: 122, 1940.

A. Place a drop of the body fluid under examination (approximately from 0.01 to 0.05 c.c.) with calibrated pipettes of the blood pipette type in the depression of a hanging-drop slide. (The liquid may be dried by air current. If the material must be shipped, the dried substance is covered with a drop (from 0.050 to 0.075 c.c.) of liquid petrolatum U.S.P. and the cover slip fastened on with a seal.) Place all slides on a clean white towel.

B. Add 0.02 c.c. of 4 normal hydrochloric acid and 0.02 c.c. of 10 per cent trichloracetic acid to the material in the depression. Break up and stir thoroughly with a glass thread. For total sulfanilamide heat over an alcohol lamp to near dryness and repeat step B.

C. Add 0.03 c.c. of 0.1 per cent sodium nitrite solution and stir the mixture thoroughly for thirty seconds with a glass rod (with amounts of free sulfanilamide in excess of 20 mg. for 100 c.c. use 0.05 c.c.).

D. Add 0.02 c.c. of a solution containing 0.1 per cent ammonium sulfamate and stir the solution thoroughly (for larger amounts of sodium nitrite use corresponding amounts of the sulfamate solution).

E. Add 0.02 c.c. of 0.4 per cent aqueous solution of N(1-naphthyl)-ethylenediamine dihydrochloride (Marshall's reagent; the solution should be kept in a dark brown bottle and only enough made up to last two days). Stir the mixture thoroughly.

F. Prepare standards (as described under standard reagents and apparatus to be used for the test) containing 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12.5, and 15 mg. per hundred cubic centimeters. If testing for sulfanilamide, use sulfanilamide in the standards; if testing for sulfapyridine or sulfathiazole, use sulfapyridine or sulfathiazole standards, respectively. Such standards are good for one week if kept in an icebox and away from direct light. The standards may be conveniently prepared by carefully diluting a larger amount.

G. Insert in the depression containing the now red solution a strip of filter paper and let the liquid spread by capillary action over one end of the paper (precipitated protein material does not interfere with the test). Compare the unknown samples with the standards. The developed color depends upon the drug used. It is necessary for the technician to know which of the sulfanilamido derivatives were used. It is important to know whether other therapeutic agents were also used. Especially with specimens of urine, a slight color varia-

tion may be noticed, depending upon various diazotizable substances. Since no fractions are to be determined and only round figures are given (2.5, 4, and 7.5 mg. per hundred cubic centimeters), no strong interference exists. The color on the filter paper will slowly fade but will hold distinctive and discernible hues for a period of days when compared with standards prepared on the same day and kept under the same conditions. Actual color charts may also be prepared to facilitate the determination at the bedside. In general for ease of reproduction the final amount of liquid in the depression should not exceed the approximate amounts of from 0.125 to 0.150 c.c.

HEPATIC DISEASE, Clinical Value of Determination of Cholesterol Esters of Blood in, Greene, C. H., Hotz, R., and Leahy, E. Arch. Int. Med. **65:** 1130, 1940.

The value for cholesterol in the blood of adults varies between 150 and 230 mg. per hundred cubic centimeters, whereas the combined cholesterol (esters) varies between 60 and 120 mg. The ratio of the combined to the total blood cholesterol is quite constant—between 40 and 52 per cent.

In this series, patients with evident hepatic damage had a decreased amount of combined cholesterol in the blood. This decrease was sometimes, but not always, associated with a decrease in the total cholesterol. The ratio per se was not of as great diagnostic usefulness as was the total amount of esters present in the blood.

In uncomplicated obstructive jaundice the combined cholesterol tends to rise in proportion to the rise in total cholesterol; but in hepatic disease the cholesterol esters tend to disappear from the blood regardless of the behavior of the total cholesterol.

In a given case a progressive decrease in the values for the combined cholesterol of the blood signifies a poor prognosis, whereas a progressive increase signifies a good prognosis.

The determination of the combined cholesterol of the blood is of great value in determining the prognosis of surgical treatment of patients with disease of the biliary tract.

TISSUE: Hamdi's Preserving Solution, Cambel, P. Arch. Path. **29:** 813, 1940.

The specimen to be preserved is fixed in a 10 to 20 per cent dilution of the 40 per cent stock solution on the market. The larger and more solid the specimen (brain, liver, large spleen, large tumor) the higher the percentage of stock solution that may be used. After the specimen has been thoroughly fixed and prepared, it is left in running tap water for at least twenty-four hours; then it is placed in a highly hypertonic (about 50 per cent) salt solution prepared with clean tap water. Here it is left for two to five days. The hypertonic salt solution penetrates the organ, drives out the formaldehyde solution, causes a slight swelling, which removes the shrinkage due to the formaldehyde solution and increases the weight of the specimen, so that the lungs, for instance, do not float when placed in the preserving fluid. It helps, moreover, to prevent the growth of molds. After the organ has been rinsed in running water, it is placed directly into Hamdi's solution, the formula of which is as follows:

Sodium sulfate (pure)	5 Gm.
Salt (pure)	100 Gm.
Clear tap water containing no organic impurities	1,000 Gm.
Glycerin	50 Gm.

The solution is clear, practically colorless. It may be yellowish if the glycerin is yellowish. Icteric organs sometimes give it an icteric tint. After a couple of years it may become slightly yellowish, especially when the specimen is exposed to much light.

A few drops of a saturated camphor solution in 96 per cent alcohol is added. The white precipitate that forms is dissipated by superficial stirring with a glass rod. Then the glass cover is sealed on the container.

If the seal loosens and molds form, and even if the color of the specimen is spoiled on account of the molds, it is sufficient to leave the specimen for half an hour to a few hours in running water and in a jar into which a few crystals of potassium permanganate have been placed. The organ is then rinsed in running water, treated with salt water or not, replaced in fresh Hamdi solution, and the jar resealed.

The advantages of Hamdi's solution are: It does not necessitate a preliminary treatment of specimens with alcohol as with most of the preserving solutions now in use. It preserves the specimens practically in their natural condition; it does not destroy the red blood corpuscles.

HYPERTENSION: I. Effect of Nephrectomy Upon, Associated With Organic Renal Disease, Schroeder, H. A., and Fish, G. W. Am. J. M. Sc. **199:** 601, 1940.

Seven patients exhibiting arterial hypertension associated with organic renal disease have been subjected to nephrectomy. Two were markedly improved, and 2 slightly improved, but all remain actually or potentially hypertensive.

This form of therapy may prove of benefit, but, it seems, only in patients in whom the existence of hypertension is of short duration and in whom arteriolar sclerosis of the other kidney is not advanced. Its use is limited, therefore, to a small number of individuals.

As criteria for selection of cases for this form of therapy the authors suggest:

1. The onset of hypertension should be known to have occurred recently (arbitrarily two years).

2. The renal lesion should be confined to one kidney and should be of such a nature that diminution of function has occurred in that kidney.

3. Renal functions, as measured by the ability of both kidneys to concentrate urine and by the test of the clearance of urea, should be within normal limits.

4. Retinitis should be absent, and changes in the caliber of the vessels of the retina should be minimal.

5. Arterial pressure should be persistently elevated.

II. ARTERIAL HYPERTENSION, The Role of the Kidney in the Pathogenesis of, Dicker, E. Am, J. M. Sc. **199:** 616, 1940.

For the kidneys to be able to cause hypertension their circulation must be restricted; all the other renal and urinary manifestations are secondary, independent, and incapable of playing a part in the production and maintenance of the hypertension.

III. ESSENTIAL HYPERTENSION: Comparison of the Hypertensive and Non-Hypertensive Phases Following Coronary Thrombosis, Gross, H., and Engelberg, H. Am. J. M. Sc. **199:** 621, 1940.

An analysis is presented of 100 autopsied cases of hypertension and severe coronary artery disease studied for the effect of the blood pressure on the subsequent course. All the patients had cardiac hypertrophy and marked myocardial damage.

Ninety patients had chronic congestive heart failure. The high incidence of heart failure is partly due to the type of patient admitted to Montefiore Hospital. The onset of heart failure frequently followed an acute coronary occlusion. This occurrence was so striking that in cases of chronic coronary sclerosis, when heart failure begins rather abruptly, a silent coronary occlusion should be suspected.

There were 24 persons with terminal acute coronary closure and in these the course of blood pressure was known for at least one year prior to death. Fifteen had hypertension persisting up to the final closure, 7 had low blood pressure for several months prior to the closure, and in 2 the blood pressure varied in the preceding year.

Analysis of the course of blood pressure subsequent to acute coronary occlusion was made. Eighteen persons had persistent hypertension (after recovery from the initial drop), 12 had permanently low blood pressure, and 10 had variable pressure. The same variations in the course of hypertension occurred when no acute occlusion could be diagnosed clinically with certainty.

Twenty-one of the 100 patients died suddenly. Many of these had the clinical picture of acute coronary thrombosis, but in only four of this group was a terminal closure found following coronary thrombosis.

The subsequent blood pressure in hypertensive patients following coronary thrombosis had no effect on longevity, or on the occurrence, severity, and duration of heart failure. Neither was there a definite relationship between the course of blood pressure and the heart weight and the duration of failure. Physiologic factors undoubtedly play a role adjusting the work of the heart to a restoration or a permanent fall in the blood pressure. These factors have been discussed.

SULFANILAMIDE, Relation of p-Aminobenzoic Acid to the Mechanism of the Action of, Woods, D. D. Brit. J. Exper. Path. **21:** 74, 1940.

Yeast extracts contain a substance which reverses the inhibitory action of sulfanilamide on the growth of hemolytic streptococci.

Examination of the chemical properties of this substance and its behavior in growth tests suggested that it might be chemically related to sulfanilamide.

P-aminobenzoic acid has high activity in antagonizing sulfanilamide inhibition.

There is strong circumstantial evidence that the yeast factor may be p-aminobenzoic acid.

SULFANILAMIDE, The Inhibition of the Action of, in Mice by p-Aminobenzoic Acid, Selbie, F. R. Brit. J. Exper. Path. **21:** 90, 1940.

The experiments described show that the therapeutic action of sulfanilamide in mice infected with streptococci can be inhibited by p-aminobenzoic acid in the same way as its antistreptococcal action is inhibited in bacterial cultures. The mechanism of its action in vivo would therefore appear to be similar, if not identical, to its action in vitro. Woods (1940) has suggested that p-aminobenzoic acid or some similar substance is essential for the growth of bacteria and that its utilization can be blocked by sulfanilamide. Many of the discrepancies which have arisen in the course of the work on the sulfanilamide drugs can be explained as due to variations in the amount of the essential substance available in the bacterial cells or in their environment (blood stream, tissues, etc.). It would also appear probable that the lag phase in the action of sulfanilamide coincides with the slow exhaustion of the essential substance.

TRICHINELLA SPIRALIS, Early Mild Infestation With, Andes, J. E., Greene, R. A., and Breazeale, E. L. J. A. M. A. **144:** 2272, 1940.

Of ten patients believed to have been infested with *Trichinella spiralis,* all but one gave a history of eating pork from one to three days before the onset.

The condition of eight of these patients was diagnosed within the first three days of the onset. The most common symptoms were malaise, abdominal discomfort, fever, headache, and edema around the eyes. All showed a definite eosinophilia and all tested gave a positive skin reaction and trichinella antigen. Administration of tetrachlorethylene was followed by complete and immediate recovery.

The two patients consulting the authors six and seven days after the onset contracted muscle pain and tenderness; the third patient showed a fairly long drawn out convalescence.

The eosinophile count of the blood was shown to rise continually during the day, being highest in the evening.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

The Author Publisher Printer Complex*

A WELL-WRITTEN booklet on the preparation of manuscript, correction of proof, and related aspects of writing and publishing a book, the title might more accurately have been "Advice From a Publisher to His Authors."

The author who has read this book will have a much clearer understanding of the problems which his publisher must solve and the decisions which he must make, many of which might otherwise have appeared unjustified.

This small work should be highly recommended to all who contemplate writing a book or an essay. It will be enjoyed equally by those who have written in the past.

Directory of Medical Specialists†

ENTERPRISING firms have in the past attempted to prepare reference catalogues of physicians in the various specialties in the United States. Some have catalogued any and all who were willing to pay for a copy of the finished book, while others have attempted some manner of selection, such as recommendation by recognized leading physicians in different localities.

None of these has been satisfactory. Those who have had occasion to investigate the qualifications of doctors in distant localities have had to rely chiefly on the sparse information available in the Directory of the American Medical Association.

With the advent of certification in the specialties after adequate examination by official boards, a satisfactory standard for inclusion in a directory of this sort has for the first time become available. The Directory of Medical Specialists includes all persons in the United States and its possessions and in Canada who are diplomats of the official boards in the various specialties. The Directory is, therefore, authentic and reliable. The Board of Editors comprises the secretaries of the several specialty boards.

Approximately 14,400 diplomas certified by the twelve special American boards and one of the two affiliate boards are listed. Cross indexing, geographical and alphabetical, is adequate.

Twice on the day of arrival of the book the reviewer had occasion to use it, thus promptly discovering that it adequately fulfills its purpose.

---

*The Author Publisher Printer Complex. By Robert S. Gill. Cloth, 76 pages, $1.00. Williams & Wilkins Co., Baltimore, Md., 1940.

†Directory of Medical Specialists. Certified by American Boards. By Paul Titus, M.D., Directing Editor. Cloth, 1,573 pages, $5.00. Published for the Advisory Board for Medical Specialties, Columbia University Press, New York, N. Y., 1940.